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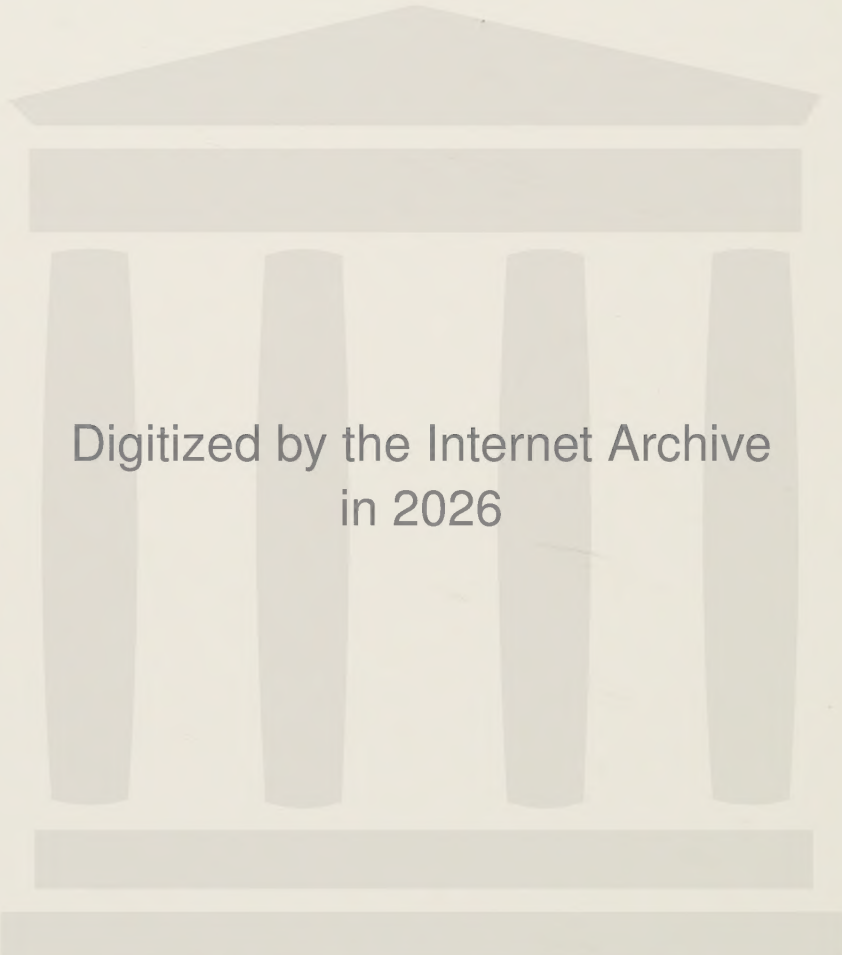
SECTION A 1972 SUPPLEMENT NO. 231

On Medullary Carcinoma of the Thyroid

BY

OTTO LJUNGBERG

MUNKSGAARD • COPENHAGEN



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FROM THE UNIVERSITY DEPARTMENT OF PATHOLOGY, GENERAL HOSPITAL,
MALMÖ, SWEDEN

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English revised by Mr. L. James Brown



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This thesis concerns medullary thyroid carcinoma with special reference to its biological nature and pathogenesis. It is based on pathological and some clinical findings in a series of medullary thyroid carcinoma, reported in this treatise and on the communications (referred to in the text by their Roman numerals) given in the following list and includes also a review of the relevant literature.

- I Ljungberg, O.: Medullary carcinoma of the human thyroid gland. Autoradiographic localization of radioiodine. *Acta path. microbiol. scand.* 68: 476—480, 1966.
- II Ljungberg, O., Cederquist, E. & von Studnitz, W.: Medullary thyroid carcinoma and phaeochromocytoma: A familial chromaffinomatosis. *Brit. M. J.* 1: 279—281, 1967.
- III Falck, B., Ljungberg, O. & Rosengren, E.: On the occurrence of monoamines and related substances in familial medullary thyroid carcinoma with phaeochromocytoma. *Acta path. microbiol. scand.* 74: 1—10, 1968.
- IV Ljungberg, O.: Two cell types in familial medullary thyroid carcinoma. A histochemical study. *Virch. Arch. Abt. A Path. Anat.* 349: 312—322, 1970.
- V Ljungberg, O.: Argentaffin cells in human thyroid and parathyroid and their relationship to C-cells and medullary carcinoma. *Acta path. microbiol. scand.* Section A. In press.
- VI Ljungberg, O. & Dymling, J.-F.: Pathogenesis of C-cell neoplasia in thyroid gland. C-cell proliferation in a case of chronic hypercalcaemia. *Acta path. microbiol. scand.* Section A. In press.

I. INTRODUCTION

Medullary thyroid carcinoma was named and defined as a distinct clinical and pathological entity by Hazard et al. in 1959 who stressed the presence of amyloid in the tumour as its most striking characteristic. As was partly propounded by Williams in 1965, this tumour has since been found to have a number of associations which gives it a unique position in the classification of thyroid neoplasms. The most interesting features are its association with phaeochromocytoma and proliferative disorders of other neuroectodermal structures, its tendency to be inherited and its character of an endocrine tumour, which may produce a number of different hormones and substances with hormonal-like effects.

The series of studies which forms the basis of this treatise and started in 1965, was

prompted by the necropsy findings made in a case of medullary thyroid carcinoma, associated with bilateral phaeochromocytomas and multiple mucosal neuromas. That year, also some familial cases of the "medullary thyroid carcinoma-pheochromocytoma" — syndrome had been observed at the department. At that time, the nature of the thyroid tumour and therefore the pathogenesis of the syndrome as a whole was enigmatic. The main purpose of the subsequent investigations was to elucidate the pathogenesis of the medullary thyroid carcinoma and its associations by studying some morphological, histo- and biochemical as well as clinical and genealogical features of the tumour and its associated disorders.

II. HISTORICAL

Occasional reports on malignant goitre with amyloid can be found in older German literature (Burk 1901, Jaquet 1906, Stoffel 1910). In 1951 Horn reported 7 cases of a thyroid carcinoma apparently belonging to this type, but he did not mention the occurrence of amyloid. Brandenburg (1954) described one case and stressed the presence of amyloid in the tumour and its metastases, but without any signs of generalized amyloidosis. He felt that the amyloid was produced by the neoplastic cells and was possibly related to colloid. In 1957, Laskowski studied 7 cases and proposed the name "carcinoma hyalinicum thyreoideae". It was not until after 1959, when Hazard et al. reported their findings, that this tumour was generally accepted as a distinct clinical entity. They described the

major histological features of the tumour, including the regular presence of amyloid in the stroma which was recognized as an important diagnostic characteristic. They stressed the importance of distinguishing it from the solid form of anaplastic carcinoma and from papillary carcinoma, from which it differed prognostically. The authors also suggested the name "medullary thyroid carcinoma" for this tumour. They admitted that this term was not ideal, since the tumour was hard rather than "medullary" in consistence. However, this name was preferred to the term "solid thyroid carcinoma" which, though more appropriate descriptively, could cause confusion with the more malignant, solid form of anaplastic carcinoma. The name "medullary thyroid carcinoma" has since been widely accepted.

III. MATERIAL AND METHODS

The pathological material of 370 surgical cases of thyroid carcinoma from the files of the Departments of Pathology at General Hospital, Malmö and at University Hospital, Lund covering an eleven-year period (1957—1967), was examined and the medullary thyroid carcinomas found were selected for further study. In addition, the material included all autopsy cases of medullary carcinoma found in our files since 1957 as well as pathological material from a number of cases received from individual pathologists who had kindly sent in material, and the author's personal cases.

The criterion for including a case of medullary thyroid carcinoma within this series, was the presence of amyloid in the tumour, or when multiple thyroid tumours were present, then in at least one of these, and/or in metastases from the thyroid tumour(s). A case was regarded as familial when one or more additional cases of medullary thyroid carcinoma was/were present in one or more blood relatives of the patient.

Attention was focused on the gross findings, especially the site and size of the carcinoma, the presence of multiple, discrete tumours within one and the same thyroid gland, the occurrence of tumours in one or in both lobes, the presence or absence of gross encapsulation of the growths as well as of vascular or other invasion. Evidence of metastatic spread to extra-thyroidal tissue and to the regional lymph nodes at the time of the

first operation was registered. In the necropsy series, the total metastatic spread was determined. The number and size of the specimens taken for histology of each primary tumour varied in extent, but was invariably sufficient to establish the diagnosis of medullary carcinoma. In the autopsy series, samples were collected from the primary tumour(s), all metastases found at gross examination, as well as from all visceral organs, columnar bone and lymph nodes in the cervical, supraclavicular, lung hilar and para-aortic regions.

The histological growth pattern of the tumours was studied. Notes were made of encapsulation and signs of vascular or other invasion, if any. Apparently unaffected thyroid parenchyma, when present, was examined for isolated microscopical tumours remote from the gross neoplasms.

The parathyroid glands, when present in the material, were also examined for histological changes.

Sections of all primary tumours and of metastatic lesions were stained with haematoxylin-erythrosin and according to van Gieson. Alkaline Congo red (Puchtler et al. 1962) and thioflavin T (Vassar & Culling 1959) were used for demonstrating amyloid. Sections stained with alkaline Congo red were also examined in polarized light. Sections stained with thioflavin T were studied by fluorescence microscopy with the equipment recommended by Vassar & Culling (1959).

Clinical information was collected by per-

sonal interviews, by telephone and, when available, from hospital records. Inquiry was also made for the occurrence of goitre and signs of phaeochromocytoma in other members of the patient's family. One family with medullary thyroid carcinoma and phaeochromocytoma has been studied in greater detail, and the preliminary findings have been reported separately (Paper II).

In addition, some cases of this series have

been used for special histochemical and chemical studies (Papers I, III, IV and V). Some of the phaeochromocytomas were also examined with histochemical methods used in the studies on the thyroid tumours. These included the argentaffin, chromaffin and iodate reactions which were carried out according to the methods described in Paper IV and the HCl-basic dye staining technique which was performed as described in Papers V and VI.

IV. RESULTS

INCIDENCE. FAMILIAL OCCURRENCE. CLINICAL PRESENTATION AND COURSE

In all, 42 cases of medullary thyroid carcinoma were collected. A brief survey of the cases is given in Table I. Medullary carcinoma was found in 27 (7 per cent) of 370 cases of thyroid carcinoma. Roughly the same proportion was found in the autopsy series, viz. 4 out of 61.

Twenty four patients were females, a sex ratio of females to males of 1.3:1.

The ages of 39 patients, at the time of diagnosis of the thyroid disease ranged from 15 to 75 years (Fig. 1). In the remaining 3 cases, where the thyroid tumours had been unknown *intra vitam* and diagnosed *post mortem*, the ages at death were 79, 83 and 87 years, respectively.

Sixteen cases were found in members of 3 apparently unrelated families. The distribution of these cases in the families is shown in Fig. 2. The progenitor of the American branch in the pedigree of Family I emigrated from Sweden to USA in 1901 with her 4 children. She later had an additional 4. This part of the family has been investigated by Drs K. Melvin, H. Miller and A. Tashjian (see Melvin et al. 1971).

The first manifestation of the thyroid tumour reported by the patients was nodular enlargement of the thyroid gland in 34 cases and enlargement of the cervical lymph nodes

in 4 (Nos 23, 26, 29 and 39). In 3 patients (Nos 17, 30 and 39), the onset of the neck tumour was accompanied by severe diarrhoea and heavy loss of weight and in another patient (No 38) by hyperparathyroidism with elevated serum calcium values and recurrent stones in the urinary tracts. In 4 of the familial cases (Nos 4, 7, 8 and 9) the thyroid tumours were discovered at the investigation of the family. In 3 cases (Nos 2, 40 and 41), the thyroid neoplasms were found incidentally at autopsy. One tumour, less than 5 mm across, was also found incidentally in a gland from a patient (No 33) operated upon for toxic goitre.

The duration of the goitre as reported by the patient is given in fig. 3. Thyroid tumour had been detected or suspected clinically in 35 patients. Hyperthyreosis was the pre-operative diagnosis in one patient (No 33). In 4 cases, the primary clinical finding had been enlargement of cervical lymph nodes (Nos 23, 26, 29 and 39). The thyroid tumours had not been known *intra vitam* in the remaining 3 patients (Nos 2, 40 and 41).

Twenty patients had undergone total thyroidectomy, accompanied in 2 by neck dissection on the same side as the site of the primary tumour and in 5 by bilateral neck dissection. In some of these patients, the surgical treatment had been performed in several seances, occasionally over a period of several years. Lobectomy or subtotal thyroidectomy was the principal operative procedure in 11

Table I. Clinical and pathological findings in 42 patients with medullary thyroid carcinoma.

Case No.	Age at presentation and sex	Familial occurrence proven	First sign of thyroid disease	Diagnosis of medullary thyroid carcinoma	Comment
1*	75	F +	Goitre	Surgical	Operated for papilloma of the bladder
2*	79**	M +	None	At necropsy	Bilateral phaeochromocytoma. Urinary tract infection with uraemia
3	42	F +	Goitre	Surgical	History of nephrolithiasis. Cutaneous fibroma-like tumours. Multinodular enlargement of right adrenal medulla found at autopsy
4*	52	F +	Goitre	Surgical	Phaeochromocytoma in right adrenal. Parathyroid hyperplasia. Carcinoma of the breast. Cutaneous fibroma-like tumours
5	39	F +	Goitre	Surgical	Phaeochromocytoma in right adrenal
6	50	M +	Goitre	Surgical	History of gastritis. Cutaneous fibroma-like tumours
7*	48	M +	Goitre	Surgical	History of gastritis. Cutaneous fibroma-like tumours. Café-au-lait spots
8*	44	F +	Goitre	Surgical	Phaeochromocytoma in left adrenal. Cutaneous fibroma-like tumours
9*	63	F +	Goitre	Surgical	Diffuse and multinodular enlargement of right adrenal medulla. History of peptic ulcer
10	31	F +	Goitre	Surgical	
11*	36	F +	Goitre	Surgical	Bilateral phaeochromocytoma
12	48	F +	Goitre	Surgical	Phaeochromocytoma in left adrenal
13	51	M +	Goitre	Surgical	
14*	32**	F +	Goitre	At necropsy	Two phaeochromocytomas in each adrenal. Small cyst in the pituitary gland
15*	40	F +	Goitre	Surgical	
16	45	M +	Goitre	Surgical, confirmed at necropsy	Two phaeochromocytomas in left adrenal found at necropsy
17	71	F —	Goitre and diarrhoea	Surgical	Operated for peptic ulcer
18	57	F —	Goitre	Surgical	
19	58	M —	Goitre	Surgical	
20	69	F —	Goitre	Surgical	
21	48	M —	Goitre	Surgical	Operated for perforated ulcer of the stomach
22	69	M —	Goitre	Surgical	
23	65	F —	Enlargement of cervical lymph node	Surgical	
24	29	M —	Goitre	Surgical	
25	40	F —	Goitre	Surgical	
26	69	M —	Lump below right angle of mandibulae	Surgical	
27	54	M —	Goitre	Surgical	
28	41	F —	Goitre	Surgical	

Table I - Continued

Case No.	Age at presentation and sex	Familial occurrence proven	First sign of thyroid disease	Diagnosis of medullary thyroid carcinoma	Comment
29	34	F	—	Enlargement of cervical lymph node	Surgical
30	67	M	—	Goitre and diarrhoea	Surgical
31*	56	F	—	Goitre	Surgical
32	44	F	—	Goitre	Surgical
33	50	F	—	Toxic goitre	Surgical Tumour incidental finding in diffuse, toxic goitre
34*	45	M	—	Goitre	Surgical
35*	60	M	—	Goitre	Surgical
36*	52	M	—	Goitre	Surgical
37*	63	F	—	Goitre	Surgical
38*	64	M	—	Lump in the right cervical region	Surgical History of peptic ulcer and hyperparathyroidism. Parathyroid adenoma found at operation
39	54	M	—	Enlargement of cervical lymph node, diarrhoea	Surgical, confirmed at necropsy Large ulcer of the stomach found at necropsy
40	87**	F	—	None	Incidental finding at necropsy Died from left cerebral infarction and pulmonary embolism
41	83**	M	—	None	Incidental finding at necropsy Died from urinary tract infection and bronchopneumonia
42	15	F	—	Goitre	Surgical, confirmed at necropsy Multiple mucosal neuromas since birth. Nephrolithiasis. Multiple pheochromocytomas found at necropsy

* Cases not derived from the series of 370 cases of thyroid carcinoma.

** Age at death.

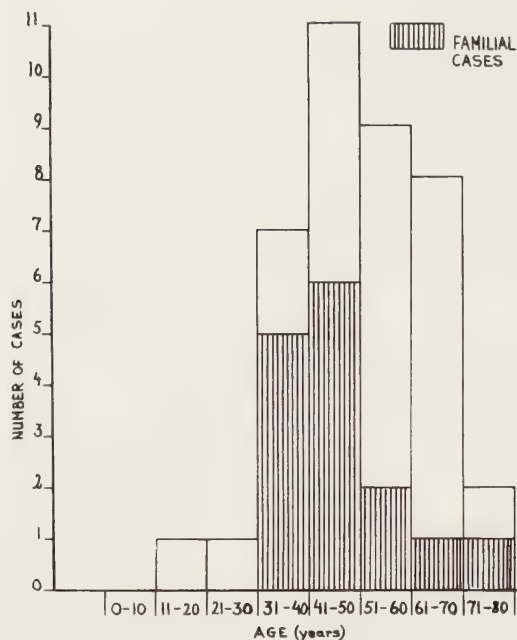


Fig. 1. Incidence of medullary thyroid carcinoma by age, in decades, at the time of the first admission to the hospital.

patients, accompanied by unilateral neck dissection in 4. In 4 patients surgery consisted only of enucleation or diagnostic biopsy and in 3 of extirpation of cervical lymph node metastases. Four patients were not operated upon.

Medullary thyroid carcinoma was the primary diagnosis made in 14 cases, in all after 1965. Six tumours had been diagnosed as undifferentiated or anaplastic carcinoma, 2 as solid carcinoma and 11 as thyroid carcinoma without any specification. Follicular and papillary carcinoma respectively, were the histological diagnoses in 2 cases. In one case, the most probable diagnosis was thought to be parathyroid carcinoma. Cervical lymph node metastases were believed to be carcinoid tumour in one case and haemangio-endothelioma or carotid body tumour in another. Hürthle cell adenoma, benign degenerated goitre, benign adenoma and solid adenoma

were the diagnoses made in the remaining 4 patients.

PATHOLOGICAL OBSERVATIONS

Gross findings

The diameter of the tumours varied from a few mm to 8 cm. In 18 cases, only one defined gross tumour was found in the gland; 13 were situated in the right lobe and 4 in the left. These tumours were encountered in different parts of the lobes and showed no predilection for any particular region. One tumour was situated in the isthmus of the gland. One or more growths of varying size were found in both lobes in another 14 cases all of which belonged to the familial group. (Fig. 4). In the remaining 10 cases — including 2 familial ones — the growths were not definable. Biopsy specimens were the only material available for study in 7 of these cases, the thyroid tumours having been considered as inoperable by the surgeon. In another 3, the entire gland had been removed and was replaced by tumour tissue and the origo of the growth(s) could not be determined.

The tumours were usually well circumscribed. Sometimes they were polycyclic in outline, occasionally irregular with gross infiltration of the surrounding thyroid tissue.

Most of the tumours were firm and had pale-brown, sometimes grey-white, slightly bulging cut-surfaces, occasionally discoloured by haemorrhage and fibrosis. Granular calcifications were seen in the primary tumours of 22 cases and in the metastases in 3. In case No 2, a 79-year-old male belonging to the familial group, necropsy showed several bony, ill-defined tumours in both thyroid lobes with extensive calcification and bone formation. The secondary deposits showed similar features.

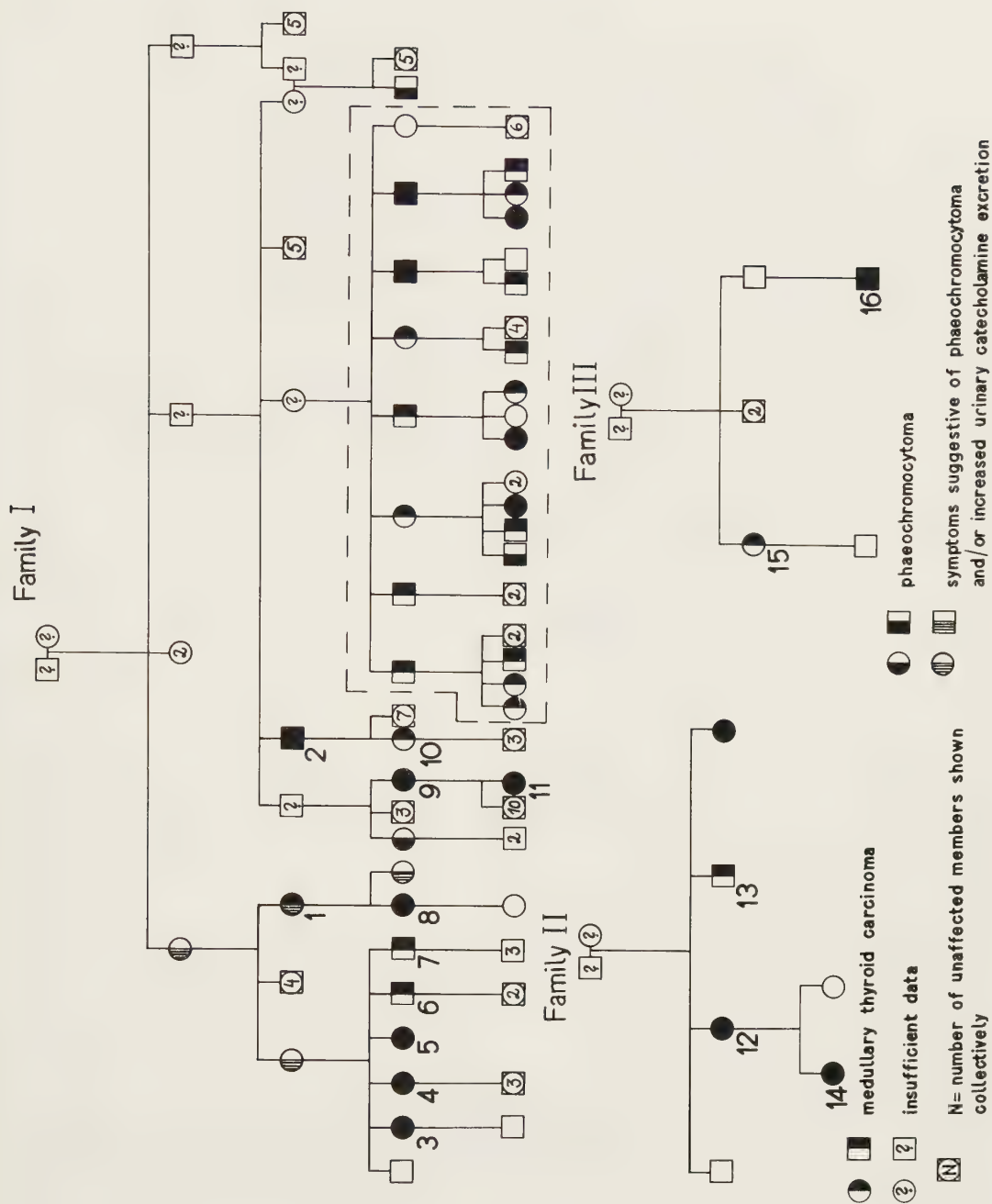


Fig. 2. Pedigrees of three families with medullary thyroid carcinoma and pheochromocytoma. Members within the area, marked by short lines in pedigree of Family I, belong to the American

branch, studied by Melvin et al. (1971) (see text). Figure below and to the left of the symbol indicates case number in the present material.

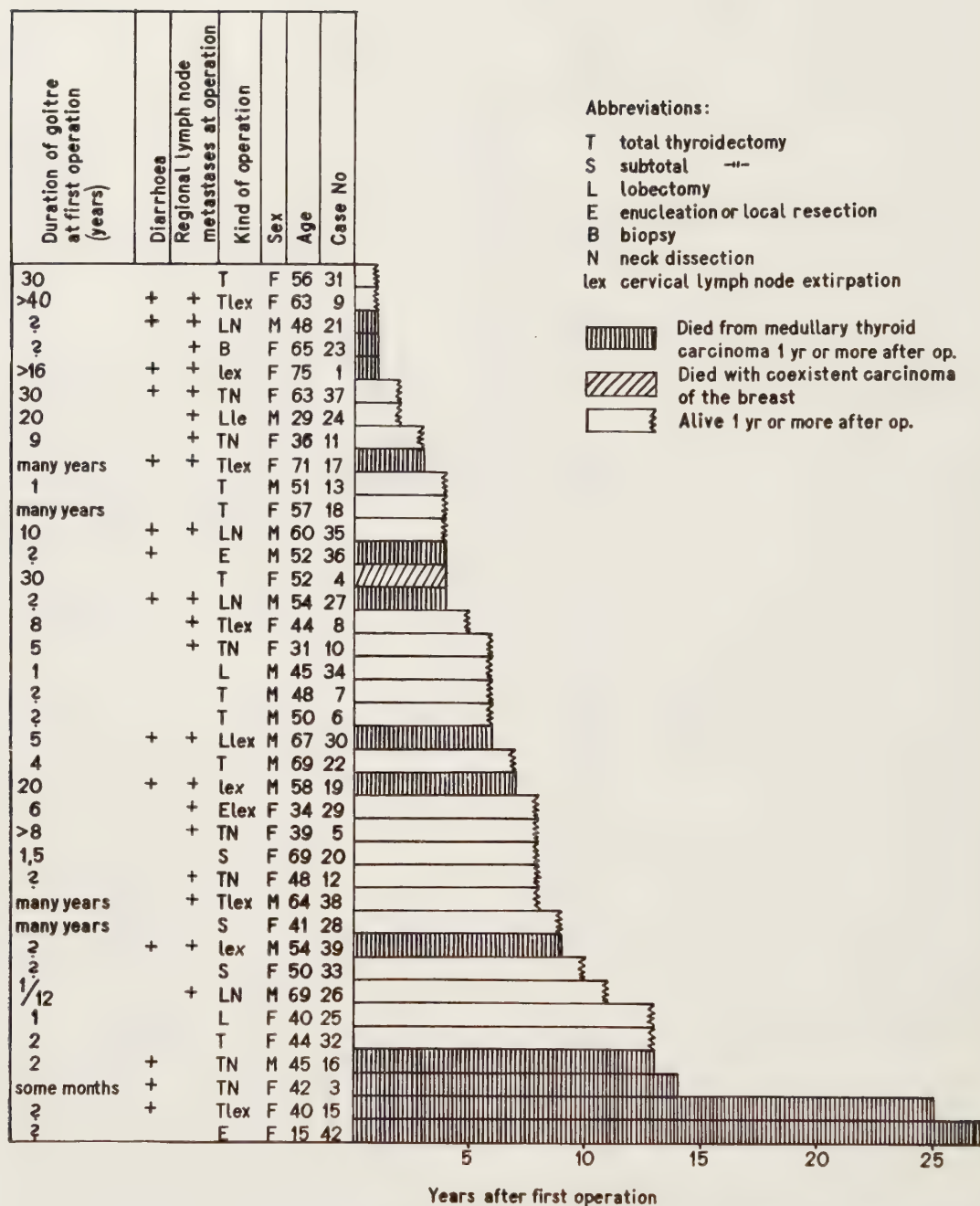


Fig. 3. Clinical and survival data in 38 operated cases of medullary thyroid carcinoma.

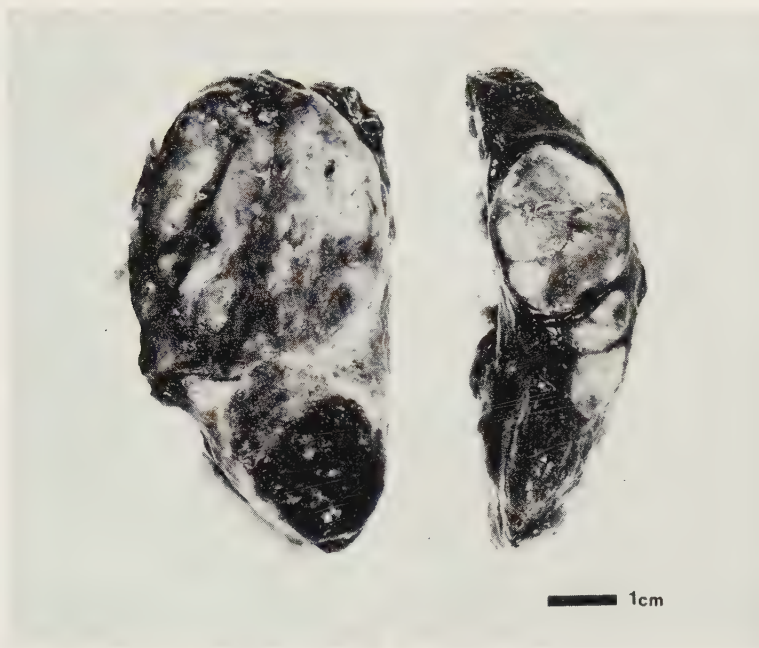


Fig. 4. Surgical specimens of both thyroid lobes, cut frontally, from a case of familial medullary thyroid carcinoma (No. 4). Both lobes are seen to be involved by gross tumours.

Metastases to the cervical lymph nodes were found at first operation and were verified histologically in 20 out of the 38 patients operated upon. Nine of these cases were familial.

Microscopical findings

Tumours. Partial encapsulation of the tumours was common (Fig. 5). It was noted in 29 cases. Infiltration of the surrounding thyroid parenchyma however, was seen in 35, including 5 with extrathyroidal infiltration. Blood vessel invasion was also a common phenomenon; it was noted in 32 tumours.

Amyloid was invariably present in at least one primary tumour. In glands which contained several, separate tumours, amyloid was, however, not a constant feature in all tumours. Several small growths were found

not to contain any amyloid. Amyloid was demonstrated in all metastases except in one necropsy case (No 39), where this substance was observed in metastases of cervical lymph nodes and liver, but not in metastases of lymph nodes from the para-aortic region and hilus of the liver. The amount of amyloid varied. It was unevenly distributed and areas completely devoid of the substance were common. No amyloid was ever detected outside the tumour tissue. The amyloid stained pink with alkaline Congo red and showed a yellow-green birefringence in polarized light (Fig. 7 d). It fluoresced grey-white when stained with thioflavin T. Its staining characteristics were the same as amyloid in various other pathological states, such as primary and secondary amyloidosis. The amyloid was intermingled with the neoplastic cells and was likewise deposited within the fibrous septa of

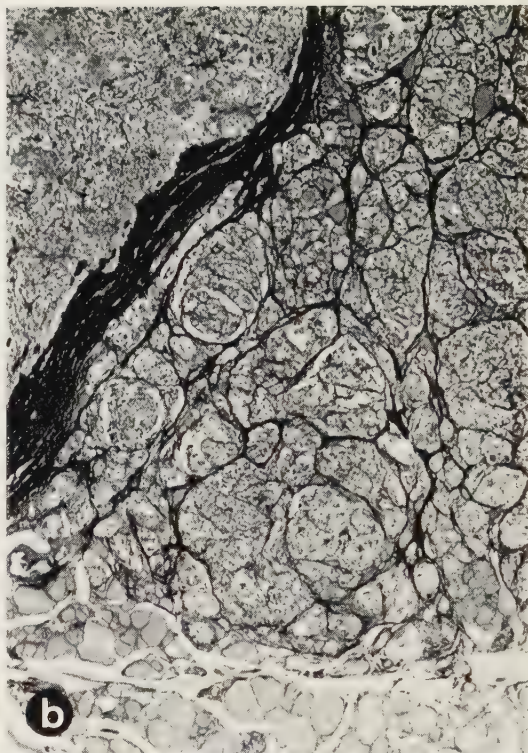
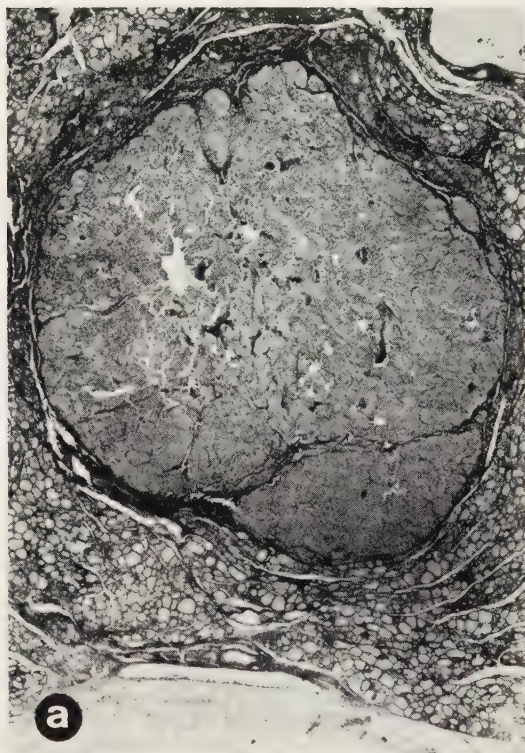
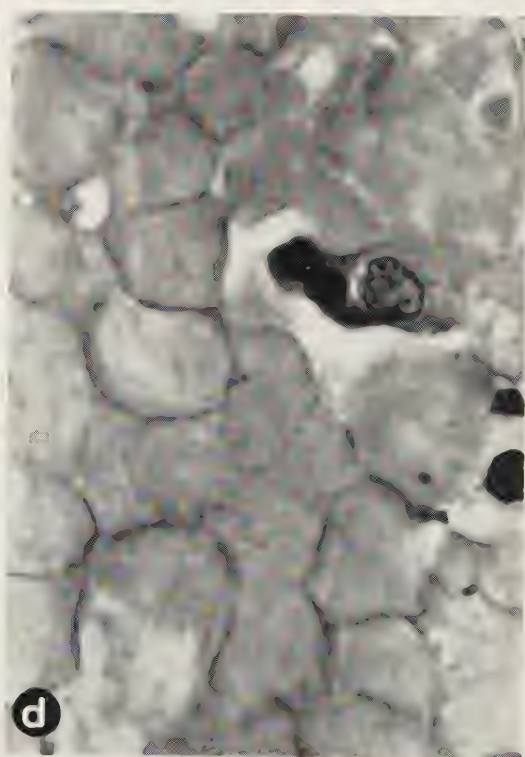
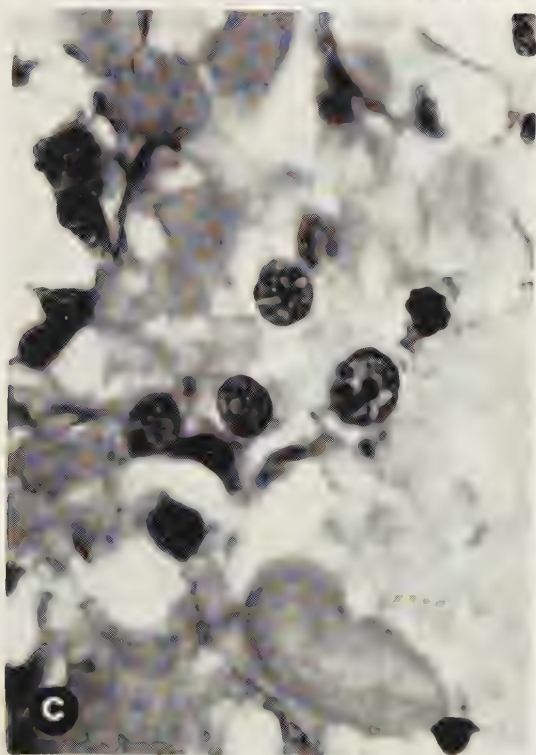
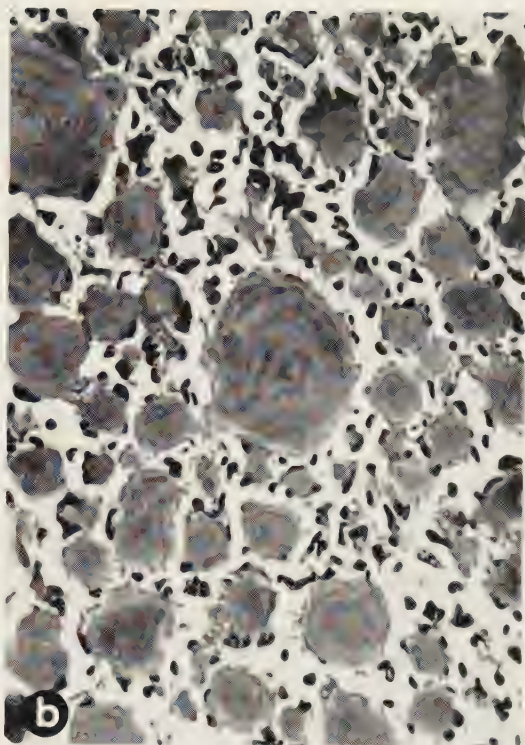
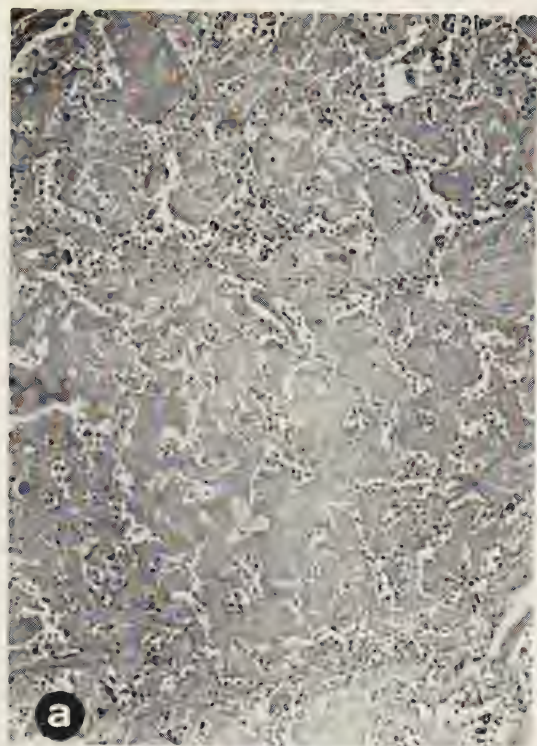


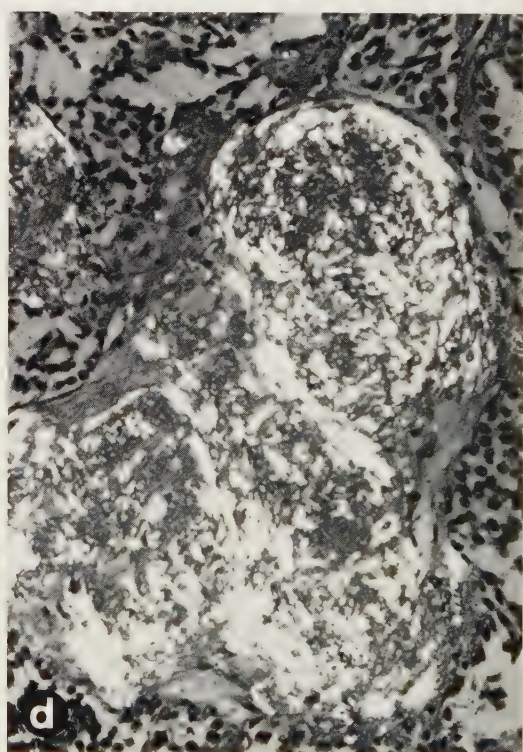
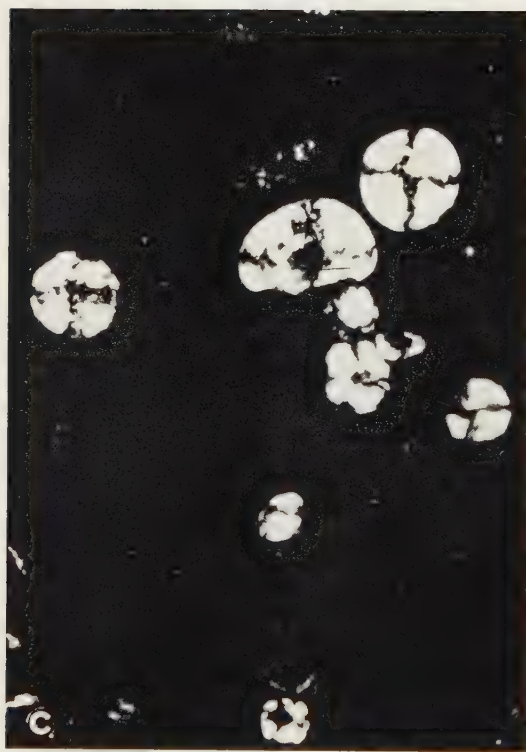
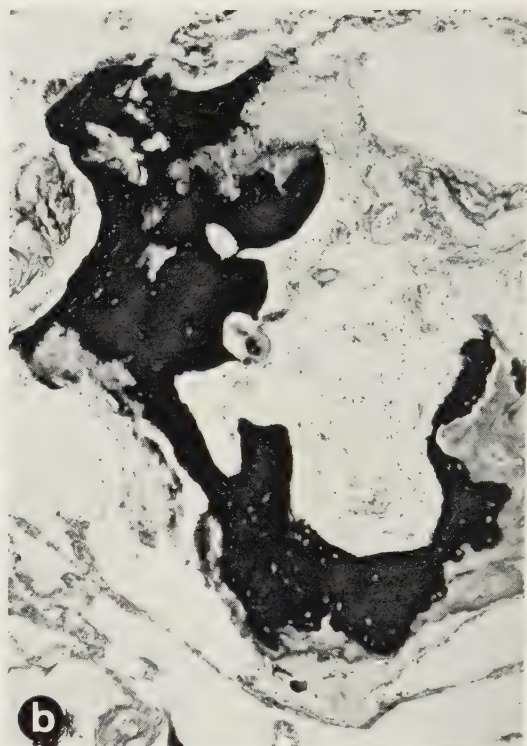
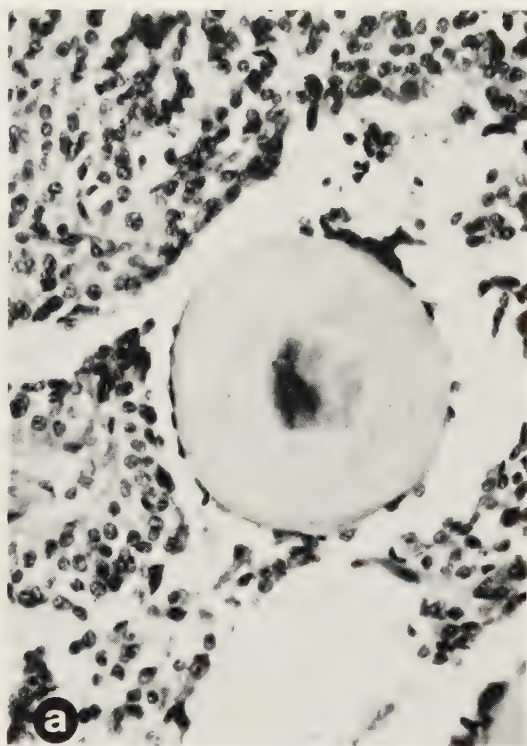
Fig. 5. a Solitary medullary thyroid carcinoma found incidentally in a case (No. 33) operated for toxic goitre. The nodule is well circumscribed. van Gieson, $\times 12$. *b* Case No. 4. Infiltration of the surrounding thyroid tissue. van Gieson, $\times 14$.

the stroma (Fig. 6). In some places the amyloid had formed small, round clumps, roughly the size of a tumour cell. These clumps were seen to have coalesced, forming larger deposits in which the contours of the "sub-units" were sometimes still visible (Fig. 6 *c* and *d*). Certain larger deposits appeared laminated (Fig. 6 *b*) as if the substance had been laid down layer by layer. Sometimes, the tumour cells were arranged in a palisade-like fashion on the surface of the amyloid masses. Disintegrating cells were sometimes embedded within the masses. Certain cells, possibly being neoplastic cells, showed small rounded clumps which appeared to be situated within their cytoplasm and had tinctorial characteristics of amyloid. Occasionally, amyloid deposits

had elicited a foreign body reaction and were surrounded by groups of foreign body giant cells. Five primary tumours contained irregularly distributed spherical bodies. They varied in size, most frequently with a diameter of about $100\ \mu$ (Fig. 7 *a* and *c*). They showed a laminated or radiate structure, were weakly eosinophil and showed the same tinctorial characteristics as amyloid. Stained with alkaline Congo red and examined in polarized light, they resembled Maltese cross-

Fig. 6. a and *b* Tumours containing abundant amyloid deposits. H.-E., $\times 75$ and $\times 300$ respectively. *c* and *d* Small clumps of amyloid coalescing to large masses where the "sub-units" are still visible. van Gieson, $\times 1120$.





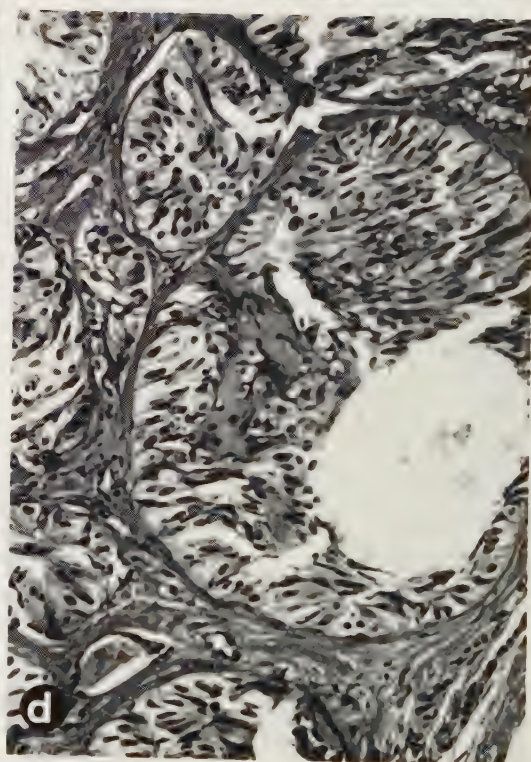
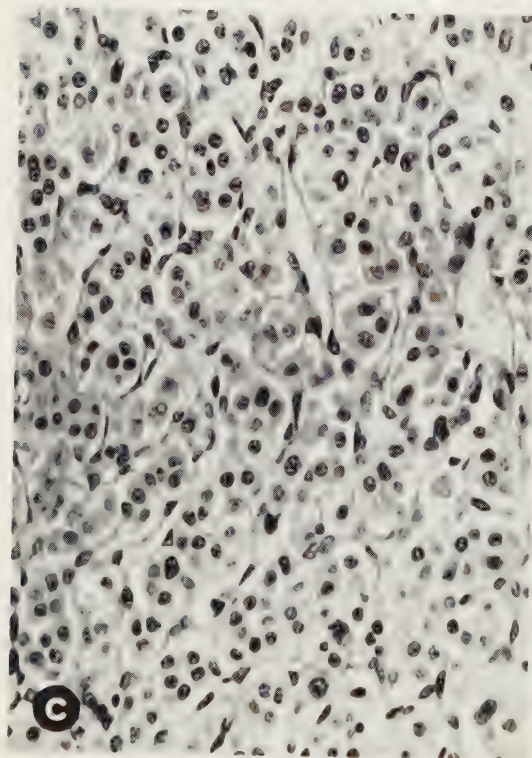
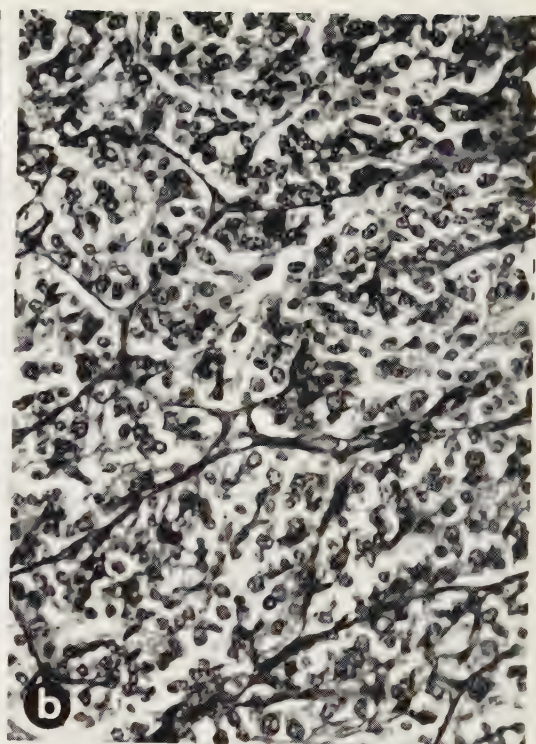
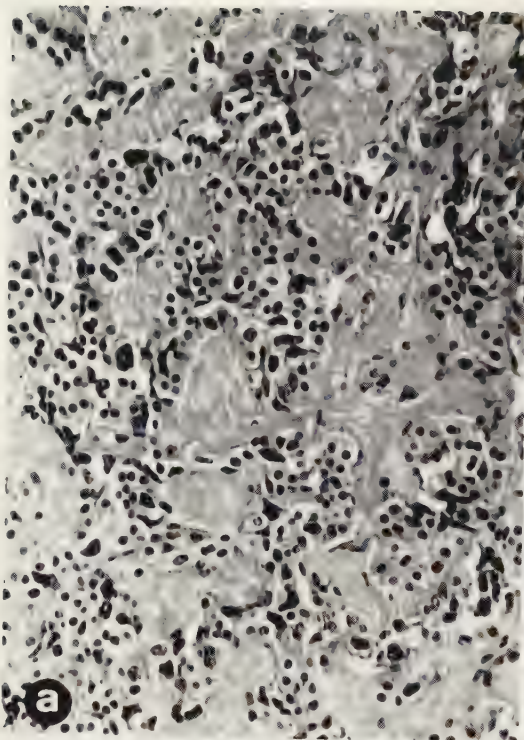
ses, similar to those of corpora amylacea (Fig. 7 c). Granular calcifications within the fibrous stroma or amyloid masses were rather common, being noticed in half of the cases. True psammoma bodies were rare.

Bone formation was present in 3 primary tumours (Cases 2, 16 and 42) and in metastases of 2 of these. The bone foci often consisted of bone trabeculae arranged in annular structures, surrounded by a zone of tumour tissue and enclosing bone marrow with blood-forming tissue (Fig. 7 b). Two of the patients had had the disease for at least 15 and 29 years, respectively. The third, a familial case (No 2), died at 79 years of age, probably after having had the thyroid disease for many years. The thyroid tumours in this case showed regressive changes with extensive replacement of the tumour cells by hyalinized connective tissue and bone.

The architecture of the tumour varied between different parts of the same tumour and also from one tumour to another. However, there was a basic type which, though varying in extent, was present in all primary tumours. This was one which could be designated the *trabecular growth pattern* (Fig. 6 a and 8 a). The neoplastic cells were arranged in irregular sheets and trabeculae, situated along intermingling, thin fibrous septa. Between the clusters of cells, there were globoid and trabecular amyloid deposits,

varying in amount. The stroma was usually scanty, but occasional broad septa divided the tumour tissue into lobular formations. Some parts with this growth pattern were not distinctly demarcated against the normal adjacent thyroid parenchyma. In the advancing zone of the tumour, smaller clusters or lobules of loosened neoplastic cells surrounded spared thyroid follicles. There was a gradual development of clusters increasing in size with progressive destruction of the normal parenchyma towards the central parts of the tumours, where thyroid follicles were no longer present. The second most common growth pattern, found in about half of the tumours, was the formation of *rounded alveoli*, composed entirely of polyhedral cells with rather abundant, weakly eosinophil, sometimes finely granular cytoplasm and usually unimorphic nuclei (Fig. 8 b and c). There were intermingled cells with dark and pale cytoplasm and often very distinct cell boundaries. In some alveoli, the cells were arranged irregularly, but often formed characteristic mosaic patterns (Fig. 8 d). Usually, there was a tendency to palisading, especially of cells in the marginal parts of the nests (Fig. 8 d). Amyloid was insignificant or absent in these clusters of tumour cells. They were separated by thin fibrous septa, occasionally containing small deposits of amyloid. These areas showed a striking similarity to a *carotid body tumour* or a *phaeochromocytoma*, (Fig. 8 c). In some areas the stroma formed the major part of the growth in which scattered nests of tumour cells were seen, thus mimicking a *carcinoid tumour* (Fig. 9 a). In one tumour, there was a gradual transition towards formation of interlacing, solid trabeculae of highly regular neoplastic cells, tightly enclosed by flattened, endothel-like cells and surrounded by interlacing spaces, sometimes containing blood (Fig. 9 b and c). No amyloid was present in areas with this type of architecture. In

Fig. 7. *a* Spherical body in thyroid tumour (van Gieson). Bodies of this type show figures similar to Maltese crosses when stained with alkaline Congo red and examined in polarized light (*c*). *b* Bone formation in pulmonary metastasis of medullary thyroid carcinoma, consisting of a semicircular structure of bone trabeculae and enclosing fat tissue with blood forming cells. Outside the bone, a small zone of partly hyalinized tumour (Case No. 42). van Gieson. *d* Birefringent amyloid masses. Alkaline Congo red. Polarized light. *a* $\times 300$, *b* $\times 75$, *c* $\times 100$ and *d* $\times 300$.



some parts in about half of the total number of tumours in this series, the cells were elongated, sometimes spindle-shaped, packed closely together and arranged in fasciculi with palisading nuclei, oriented in the same direction as the fasciculi and forming streaks and curves running in various directions. The architecture was sometimes very similar to a *neurilemmoma* (Fig. 10). Occasionally, the clusters of tumour cells were separated by small intervening patches of granular, intercellular material, which was weakly eosinophil. Amyloid was sometimes found as small clumps in thin stromal septa or between the tumour cells, but usually in only insignificant amounts. Less common patterns included *follicle-like* and *papillary structures* (Fig. 11 a and b) and areas mimicking an *angiomatous tumour*, forming large vascular channels, containing blood and lined by tumour cells.

The cytological features of the tumour cells also varied. Most of the cells were polyhedral, often with distinct plasma membranes, a varying amount of cytoplasm, which was often finely granular and eosinophil, sometimes more or less amphophil. In certain areas, the cells showed a markedly vacuolated cytoplasm (Fig. 11 c). Those parts showing a lobulated growth pattern, often contained cells, whose cytoplasm was frayed, thus giving the clusters of cells a loose, reticular appearance (Fig. 11 d). The nuclei varied somewhat in size, but were usually rounded with a fine chromatin network and small nucleoli. The nuclei often lay eccentrically in the cells. Binucleated cells were common.

Though the cells of the tumour most often displayed only a slight degree of cellular and

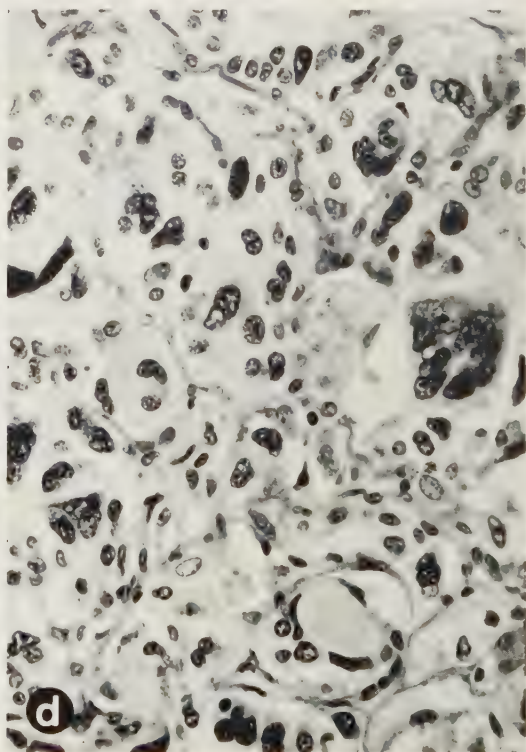
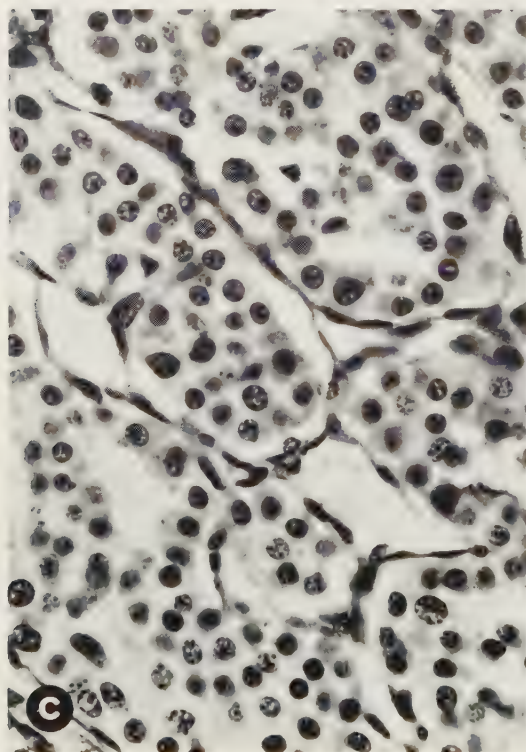
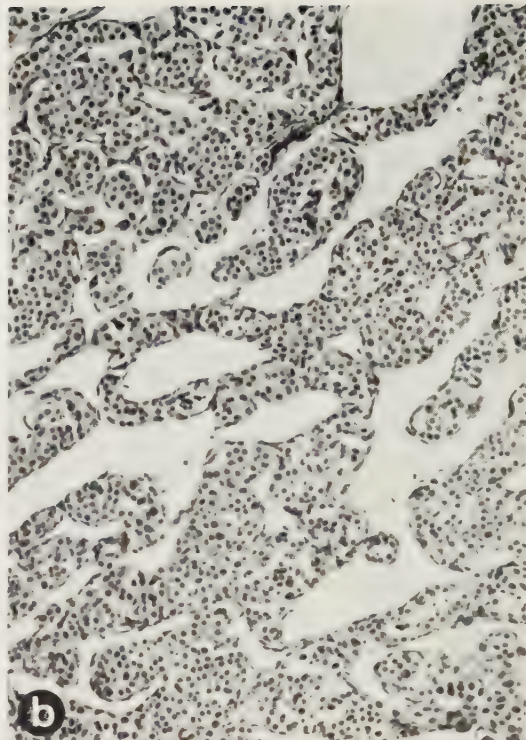
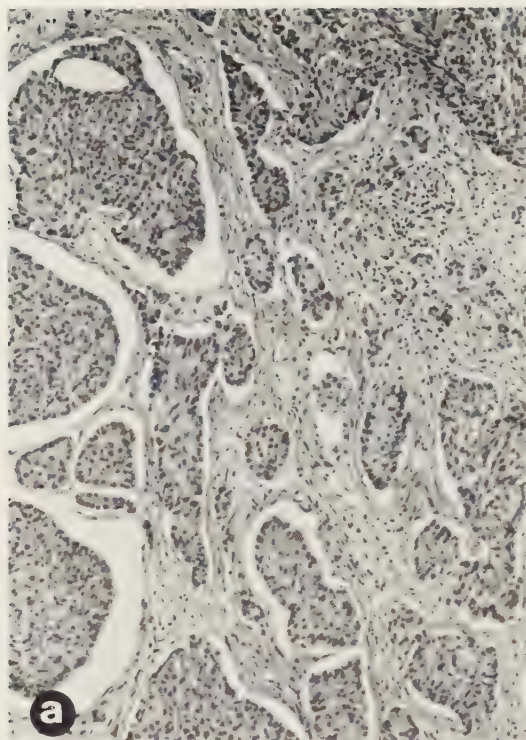
nuclear polymorphism, some areas were usually found where this was marked. In a few tumours, certain parts contained highly polymorphous cells, some of which were giant cells with single or multiple monstrous hyperchromatic nuclei. (Fig 9 d).

A striking feature of all tumours examined was the scantiness of mitotic figures. These were seen only occasionally and it is noteworthy that they were not encountered in areas with a highly polymorphous cellular pattern of the type described above.

Thyroid parenchyma, remote from the gross tumours. Material from both lobes was available for histological study in 20 cases, 14 of which were familial. Multiple microscopical clusters of epithelial non-follicular cells were found in the parenchyma of both lobes in 10 of these familial cases, all of which showed multiple gross tumours. On the other hand, each of the 6 thyroid glands, removed from patients where familial occurrence was not proven, contained only a single gross tumour in one lobe. In these cases, microscopical foci of non-follicular cells were totally absent in sections from parts of the glands not involved by the gross tumours.

The microscopical foci of cells, found in the familial cases, were situated in various parts of the glands without any special site of predilection. They were found in the stroma, between the follicles and often parafollicularly (Fig. 12). They formed different-sized clusters, some of which showed densely packed, polyhedral cells, forming mosaic-like patterns. Others had a loose, reticular appearance and were built up of loosened cells with ragged cytoplasm and irregular outer contours. These clusters of cells were usually delimited by a structure similar to a basement membrane. Occasionally, however, they were only diffusely outlined.

Fig. 8. Variants of histological growth pattern in medullary thyroid carcinoma. Note the close similarity of the thyroid tumour in Fig. 8c and the pheochromocytoma in Fig. 13c. H.-E., a $\times 220$, b $\times 260$, c $\times 300$ and d $\times 180$.



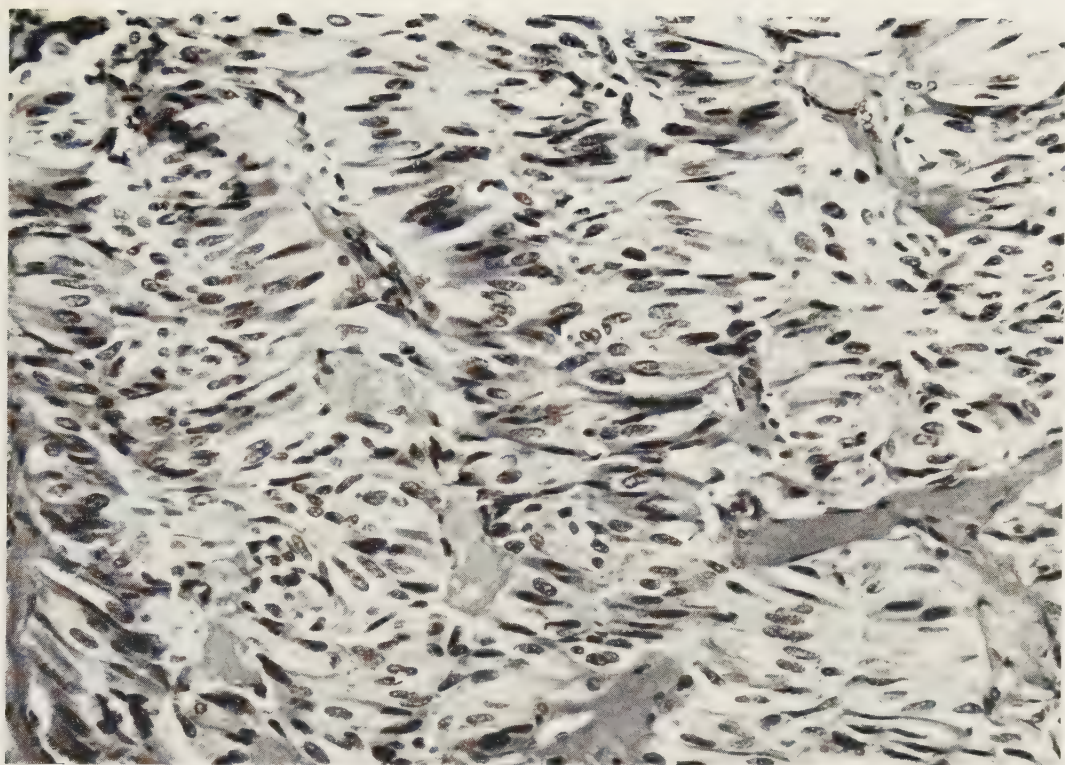
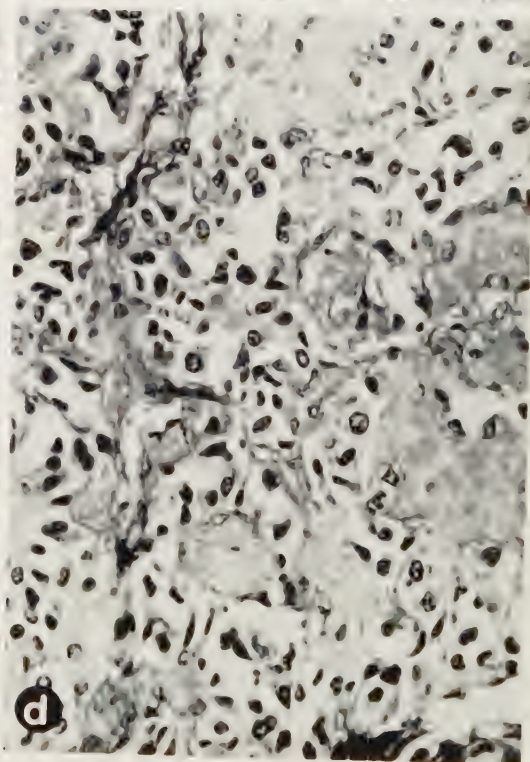
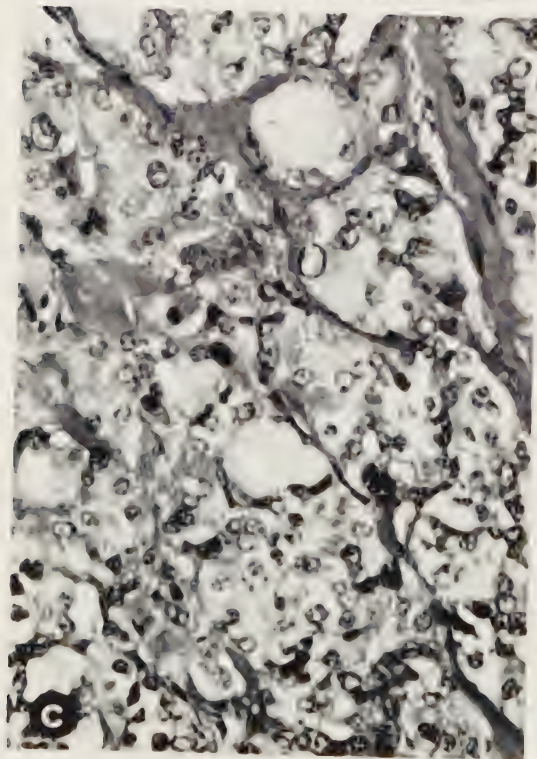
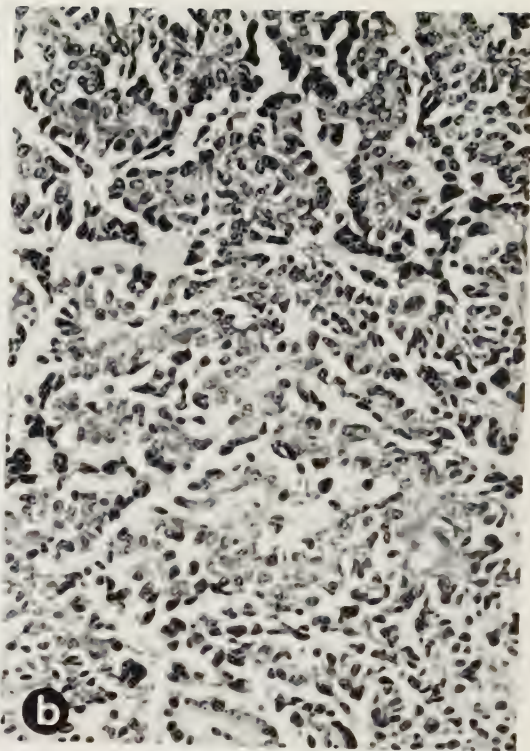
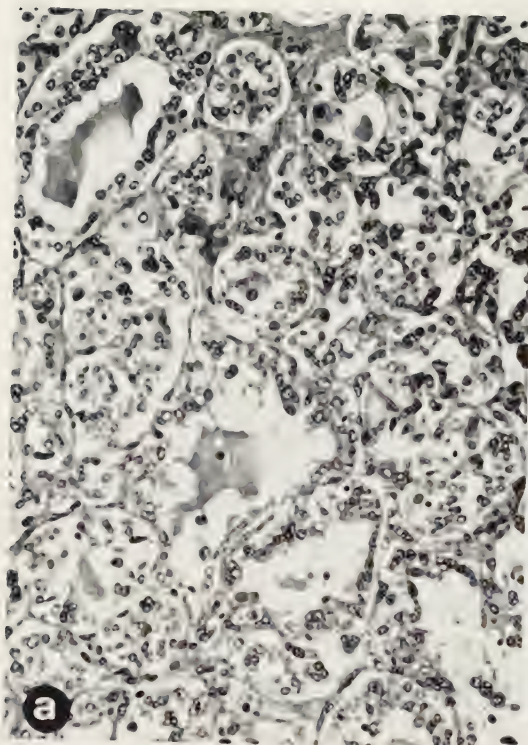


Fig. 10. Medullary carcinoma composed of palisading spindle-cells. Small amounts of amyloid are present in connective tissue septa. H.-E., $\times 260$.

Besides these interfollicular foci of epithelial cells, there were also clusters of cells, which were different from the follicular epithelial cells and which appeared to be situated within the follicular walls, sometimes causing crescent-like thickenings of these walls (Fig. 12). In some places, minute groups of non-follicular cells were bulging as drops into the lumen of the follicle. Small parafollicular clusters of cells were occasionally seen to have

coalesced, forming an annular structure surrounding a degenerating true follicle with an intact basement membrane (Fig. 12 a). Within one and the same gland, there were sometimes several, isolated nodules of increasing size, composed of more or less completely coalesced smaller clusters of cells and causing progressive destruction of the normal thyroid follicles (Fig 12 d). Amyloid was present in increasing amounts with increasing size of the nodules, although this material was not a constant feature. With increasing size of the nodules, there was also a progressive derangement of the lobulated architecture of the growths and a gradual transformation to trabecular, spindle-cellular or other growth patterns, seen in the gross tumours. Some gross tumours, however, con-

Fig. 9. *a, b and c* Medullary thyroid carcinoma with structures mimicking carcinoid tumour, sometimes forming interlacing, solid trabeculae of highly regular cells, tightly enclosed by flattened cells and surrounded by spaces which may contain blood. *d* Marked cellular and nuclear polymorphism in medullary carcinoma. van Gieson, *a* $\times 100$, *b* $\times 120$, *c* and *d* $\times 300$ respectively.



tained areas where the lobular pattern was still preserved. Usually, such parts showed no distinct demarcation against normal neighbouring thyroid parenchyma, where small clusters of neoplastic cells were seen surrounding the spared thyroid follicles (Fig. 5 b).

Histochemical studies. The results of these investigations are reported separately in Papers III, IV and V.

PATHOLOGICAL AND CLINICAL ASSOCIATIONS

Parathyroid changes

One or more parathyroid glands or islands of parathyroid tissue were identified on gross examination or in histological sections of the thyroid specimens in 14 cases.

A single adenoma was found in 2 cases. One of these was a necropsy case (No 14), belonging to the familial series and also having multiple, bilateral adrenal phaeochromocytomas. The parathyroid adenoma, approximately 5 mm in diameter, was composed of chief cells and oxyphil cells. There were no signs of osteitis fibrosa in sections from columnar bone.

The other patient (No 38) was a 64-year-old male with a negative family history. This patient had shown clinical signs of hyperparathyroidism with serum calcium levels around 6.1 mEq/l. He had suffered from renal stones for several years and had had a duodenal

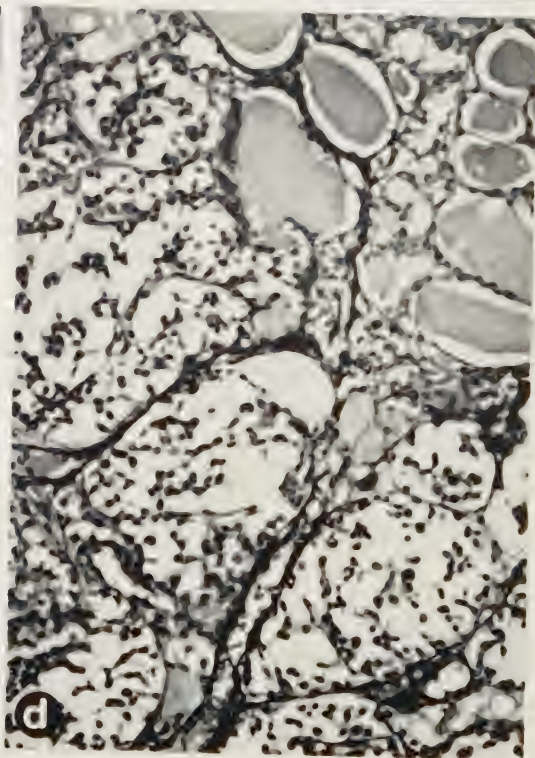
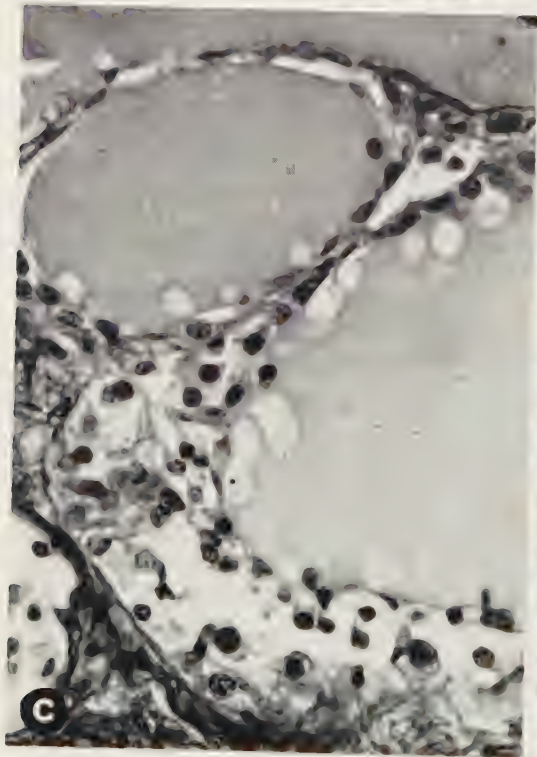
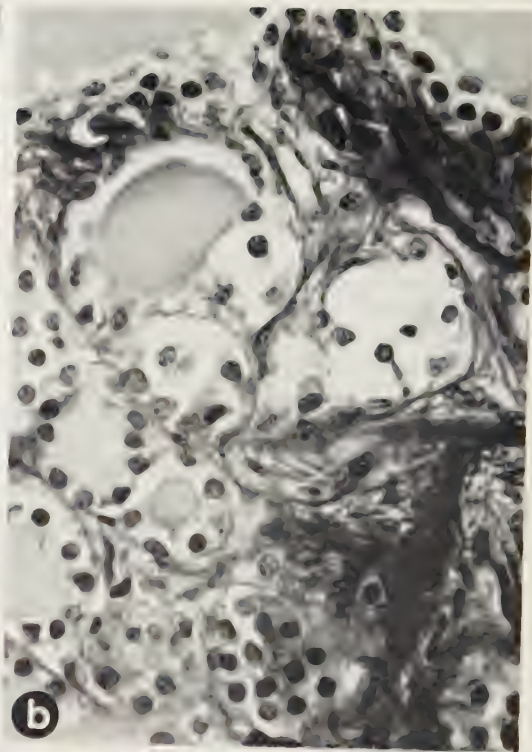
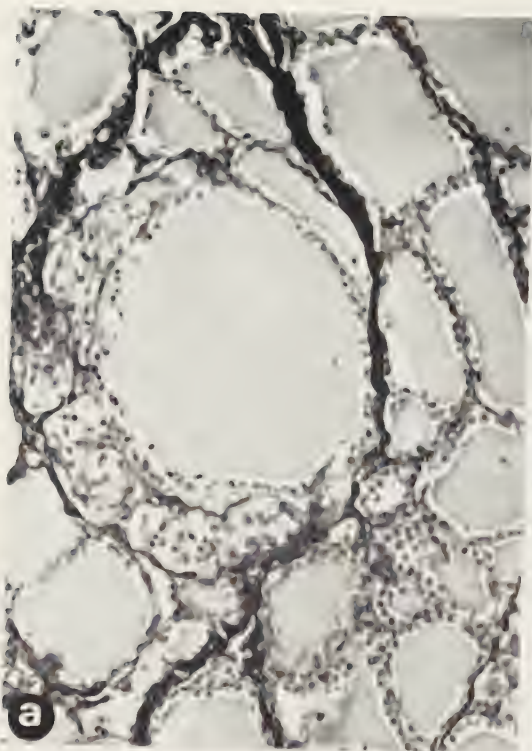
peptic ulcer many years before the operation. At operation, a parathyroid tumour weighing 130 g was removed, as well as a rounded tumour in the right thyroid lobe, which was found to be a medullary carcinoma. The parathyroid tumour was a benign adenoma consisting of oxyphil cells and a minor proportion of chief cells. Serum calcium returned to normal postoperatively.

In a third case (No 4), belonging to the familial series, an enlarged parathyroid was found on histological examination of the thyroid specimens. It consisted of chief cells and it could not be determined whether the enlargement had been due to adenoma or hyperplasia. In the remaining 11 cases, the parathyroid tissue found showed no remarkable histological features.

Phaeochromocytoma

So far, phaeochromocytoma, coexisting with thyroid cancer, has been diagnosed histologically in 11 cases (Table I). Ten of these were familial, while in the eleventh (No 42), information about the family was incomplete. Phaeochromocytoma without medullary thyroid carcinoma was present in 3 family members (See fig. 2). No extra-adrenal tumours were found. In 7 cases, the adrenal tumours were bilateral and/or multiple, including one case where phaeochromocytoma was also present in an accessory adrenal gland. Single phaeochromocytoma was found in 4 patients. In 7 of the familial cases operated for phaeochromocytoma with or without associated medullary thyroid carcinoma, there had been acute attacks of cardiovascular disease for varying periods before the surgical removal of the adrenal tumours. These "episodes" generally started with bradycardia, paraesthesia and pallor of the face, hands and feet, severe pounding headache and dyspnoea and

Fig. 11. a and b Areas in the thyroid tumour showing follicular and papillary structures. *c* Part consisting of large vacuolated tumour cells. *d* Clusters of loosened cells with frayed cytoplasm and intermingled with small clumps of amyloid. H.-E., *a* and *b* $\times 180$, *c* and *d* $\times 300$ respectively.



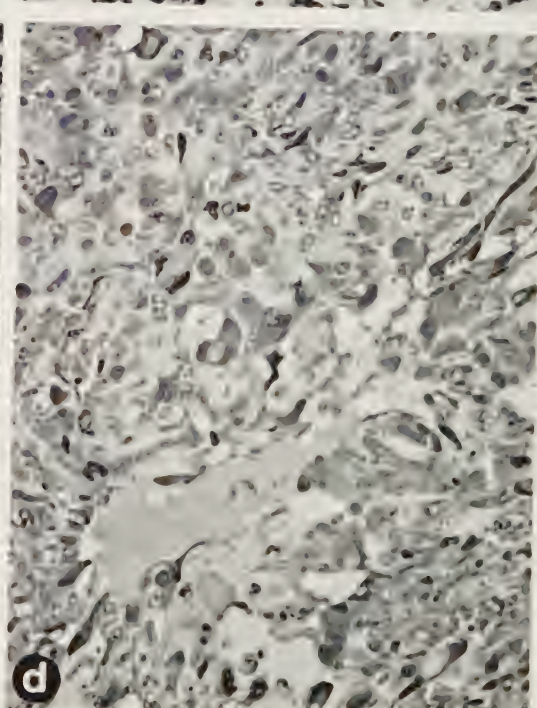
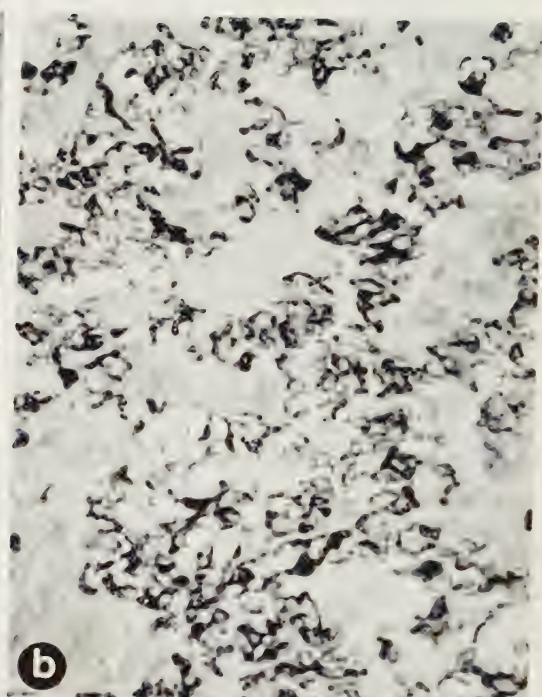
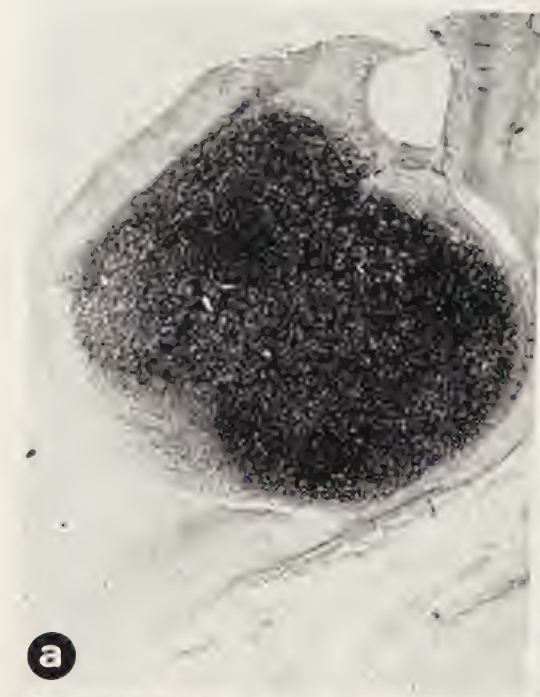
were suddenly followed by tachycardia, a sensation of heat, and reddening of the peripheral parts of the body and in some cases, also a tendency to perspiration. This complex of symptoms showed a remarkably constant pattern in all these patients, though the severity and frequency of the "episodes" varied. None of the patients had had substandard hypertension. In one patient (No 8) the attacks were reported to have started about 10 years before the onset of the goitre, in another (No 4), about 20 years after the thyroid disease had become apparent. In the remaining 4, the relation between the onset of the two diseases was unknown. The thyroid operations were invariably performed before the removal of the adrenal tumours. In one patient the severity and frequency of the cardiovascular attacks were reported to have increased after the thyroid operation, in 3 patients no change had occurred and in one, some improvement was experienced.

Grossly, the size of the adrenal tumours ranged from less than 1 cm up to 15 cm in diameter, the largest weighing 1,020 g. The small tumours were solid and soft, with greyish, sometimes haemorrhagic cut-surfaces. The large tumours were usually partly cystic, showing degenerative changes with fibrosis and calcification. In two cases (Nos 3 and 9), the adrenal medulla was diffusely increased in size and in addition, contained several more or less demarcated nodules, up to 0.5 cm in diameter. The histological structure of

the tumours was characteristic of pheochromocytoma. The growth pattern was less variable than that of the thyroid tumours and dominated by a trabecular or alveolar type of architecture. The similarity to medullary thyroid carcinoma was sometimes striking but the tumours did not contain any amyloid (Fig. 13 c). In addition, the cells of the tumours were generally somewhat larger than the cells in the thyroid tumours, and giant tumour cells were common. No structures suggesting ganglioneuroma were found. There were no signs of malignancy, such as metastatic spread of the tumours. In the 2 cases with diffuse enlargement of the adrenal medulla, there were also nodules of large polyhedral cells, embedded in the hyperplastic medullary tissue. Whether these nodules should be regarded as nodular hyperplasia or minute pheochromocytomas was debatable.

Some tumours, analysed for monoamines, contained noradrenalin, adrenalin and dopamine in amounts generally found in tumours of this kind. In addition, there were small amounts of 5-hydroxytryptamine (for detailed information, the reader is referred to Paper III). The majority of the tumour cells contained chromaffin material and many cells showed a brownish cytoplasmic granular material after oxidation with potassium iodate. The tumours contained cells, whose cytoplasm displayed a distinct metachromasia when stained with the HCl-basic dye technique after fixation in glutaraldehyde-picric acid-acetic acid-mixture (according to Solcia et al. 1968), using Azur A and pseudoisocyanin or when stained with cresylviolet without acid treatment (Fig. 13 d). Scattered argentaffin cells, as shown with the method of Masson-Hamperl, were also found in the adrenal tumours (Fig. 13 a and b). These cells closely resembled the argentaffin "spider-cells", previously reported often to occur in medullary thyroid carcinoma (Papers IV and V).

Fig. 12. Thyroid gland with familial medullary carcinoma. Remote from the gross tumours are multiple, varied-sized clusters of proliferating epithelial cells often with vacuolated cytoplasm and situated between the follicles or partly within the walls of the follicles (a, b and c), illustrating various stages of development to the true tumours (d) with a definitive destruction of the normal tissue. van Gieson, a $\times 180$, b $\times 450$, c $\times 450$ and d $\times 180$.



Other associations

Mucosal neuromas were present in one patient (Case No 42). This case has been reported previously (Ljungberg 1966), but the essential features deserve recapitulation here.

The woman had had multiple neuromas, up to 1 cm in size, on the free margins of the upper eye-lids, lips and marginal parts of the tongue, which were reported to have been present since birth. She had always been thin with slender limbs and she had a peculiar facies showing coarse features with thick lips. At 15 years of age, she was treated surgically for a thyroid tumour which had primarily been diagnosed as a benign adenoma, but on re-examination was found to be a medullary thyroid carcinoma. She had had nephrolithiasis and, since the age of 30, radiographically demonstrable multiple calcified foci in the lungs. Serum calcium levels had been elevated on several occasions. She also developed a yellow-white, ringlike opacity in the marginal part of the cornea. Terminally, the patient showed hyperglycaemia and died in a hypotensive crisis, 42 years old. *At necropsy*, there were widespread calcified deposits of medullary thyroid carcinoma, multiple pheochromocytomas in both adrenals and in an accessory adrenal gland on the left side. There were renal stones. No changes were noted in

the gastro-intestinal tract. The thyroid region showed fibrosis and extensive growth of tumour, and the parathyroids could not be found. Specimens for histological examination of the corneal changes were not obtained. Histologically, the tumours of the lips were pedunculated nodules situated in corium and built up of thickened, convoluted nerve bundles, surrounded by a thickened perineurium. No nerve cells were encountered within these lesions.

In one familial case (No 7), the patient had multiple *café-au-lait-like skin spots* on the trunk, and a few also on the limbs. Five patients, belonging to the same family, showed *multiple cutaneous tumours*, resembling fibromas, up to 1 by 1 cm in size, and mostly situated on the face and limbs. Some tumours of this kind had been excised but unfortunately not saved for histological diagnosis.

Diarrhoea was present in 14 (30 per cent) patients of the forty-two cases in this series (Fig. 3). It was usually reported to have been severe, sometimes refractory to treatment, and characterized as numerous watery, frothy stools frequently accompanied by severe loss of weight. Eleven of the patients complaining of this symptom, died, with *intra vitam* or necropsy evidence of dissemination of the thyroid carcinoma. In general, the severity of the diarrhoea was proportionate to the extent of progression of the malignant disease. In one patient (No 39), diarrhoea had been the predominating clinical feature. In this patient, hemi-hepatectomy with removal of a large part of metastatic tumour was followed by cessation of the diarrhoea and a temporary clinical improvement, with some gain in weight.

Two patients had been surgically treated for *peptic ulcer* and at necropsy, another had a large penetrating ulcer in the antrum, with signs of haemorrhage to the gastro-

Fig. 13. a Small adrenal medullary tumour in case No. 11, stained by silver precipitates after treatment according to Masson-Hamperl's argentaffin silver method. *b* Many adrenal medullary cells show cytoplasmic argentaffin material (Masson-Hamperl). *c* Small-alveolar growth pattern in pheochromocytoma from case No. 11 which may closely mimic medullary thyroid carcinoma (see Fig. 8*c*). van Gieson. *d* Another part from the same tumour, stained with cresyl violet. Some cells show cytoplasmic metachromasia (cells with dark cytoplasm), similar to cells in medullary thyroid carcinoma. *a* $\times 9.5$, *b* $\times 180$, *c* $\times 200$, *d* $\times 180$.

intestinal tract. Two patients had been treated conservatively for a radiologically verified duodenal ulcer and another 2, had received medical treatment for symptoms of gastritis.

A coexisting *carcinoma of the breast* developed in one female patient (No 4). Histologically, it was a scirrhus carcinoma and there were metastases in regional axillary lymph nodes.

SURVIVAL DATA. SPREAD OF THYROID TUMOUR

Survival data about 38 cases subjected to surgical treatment are summarized in Fig. 3. Eighteen of the forty-two patients died. In 14 patients, there was evidence of disseminated carcinomatosis *intra vitam* or at necropsy. Necropsy was performed in 7 cases; the sites of deposits of thyroid carcinoma are given in table II. The main cause of death in one case (No 16) was local growth with infiltration into the mediastinum, and in 2 cases (Nos 39 and 42), extensive metastases in distant organs. Necropsy of one patient

(No 14), who belonged to the familial series and suddenly died in a hypertensive crisis, revealed multiple phaeochromocytomas in both adrenals and multiple growths of medullary thyroid carcinoma in both thyroid lobes, but without any signs of extra-thyroidal spread. Another (No 2), also belonging to the familial group, died 79 years old from uraemia on the basis of prostatic hyperplasia and severe urinary tract infection. In that case there had been no anamnestic or clinical evidence suggesting a thyroid tumour or phaeochromocytoma. A daughter (No 10) of the patient was known to have been operated for medullary thyroid carcinoma. Judging from the genealogical information available in this case (see Fig. 2, Family I), the patient was affected with the disease. This assumption was verified at necropsy in that multiple growths of medullary carcinoma were found in both thyroid lobes, as well as bilateral adrenal phaeochromocytomas. There were metastases from the thyroid tumours in the cervical lymph nodes and a minute deposit (2 by 2 mm) in the liver. The thyroid tumours and their secondaries showed extensive regressive changes, the tumour cells having small, pyknotic nuclei and large parts of the tumour tissue had been replaced by hyalinized fibrous tissue with bone formation and extensive calcifications, giving the impression that these tumours had ceased to progress.

Death of the remaining 2 patients (Nos 40 and 41) had been caused by unrelated conditions (see table I). Single thyroid tumours, 3 and 0.4 cm in diameter, respectively, were found incidentally at necropsy in these 2 cases, and there was no extra-thyroidal spread.

Table II
Sites of deposits of medullary thyroid carcinoma found at necropsy of seven cases.

	Case No.	Total
Cervical lymph nodes	2, 16, 39, 42	4
Lung hilar lymph nodes	16, 42	2
Para-aortic lymph nodes	16, 42, 39	3
Liver	2, 16, 39, 42	4
Lungs	16, 42, 39	3
Bone	16, 42	2
Pleurae	16, 42	2
Myocardium	42	1
No extra-thyroidal spread	14, 40, 41	3

V. DISCUSSION

INCIDENCE. CLINICAL FEATURES AND COURSE

Medullary thyroid carcinoma is not very common. In major published series of thyroid carcinoma, the medullary type comprises 3.5 to 11.9 per cent (Table III). The figure in the present series is about 7 per cent. It is difficult to interpret these figures, since the incidence may vary from one geographical area to another, as is the case for thyroid carcinoma in general.

The sex distribution (females : males) in the above series has varied from 0.9 to 3.7 : 1. In the present material it was 1.3 : 1. The average sex distribution, all series taken together, is about 1.5 : 1. This differs from that of papillary and follicular carcinoma, which are 2 or more times as common in the female as in the male, but is similar to that of anaplastic carcinoma (Woolner et al. 1961). The sex distribution of proven familial medullary carcinoma with or without associated phaeochromocytoma, appears to be almost the same as for the sporadic form. Out of 110 familial cases collected from the literature (see Appendices I and II), 64 were seen in females, a ratio of females to males of 1.4 : 1.

The age of the patients at the time of the first operation for the thyroid tumour varied considerably in the present series. The youngest was 15 years old, and the oldest 75 years, the majority being between 40 and 50

years. It is difficult to determine the average age at onset of the thyroid disease. As seen in Fig. 3, the duration of the thyroid enlargement, as experienced by the patient, varied widely, some patients having felt a thyroid nodule since childhood, others not having noticed any nodule before they had reached an old age. In this respect, there does not seem to have been any difference between the proven familial cases and the others where familial occurrence was not found.

The course of the disease had fluctuated considerably. Sixteen patients out of 27 had been aware of an almost stationary thyroid enlargement for more than 5 years — two of these for more than 40 years — before surgical treatment, while, in the remaining 11 cases, this symptom was reported to have been present for less than 5 years before operation. The thyroid enlargement was said to have been "silent", without tenderness or pain. Some patients had sought medical advice merely for cosmetic reasons. Though the preoperative course was often slow, metastases were verified histologically in the cervical lymph nodes at the time of diagnosis in about 52 per cent. The true proportion is presumably still larger, as pointed out by Williams et al. (1966). This finding is consistent with the generally accepted opinion that this tumour metastasizes early to regional lymph node stations, but further extra-thyroidal dissemination often appears to occur rather late. The necropsy findings in the 79-year-old man

Table III. Frequency and sex distribution of medullary thyroid carcinoma in major published series

Authors	Total number of thyroid carcinoma	Number of medullary carcinoma	Sex ratio Female : Male
Hazard et al. USA 1959	600	21 (3.5 per cent)	2 : 1
Woolner et al. USA 1961	885	57 (6.4 per cent)	1.3 : 1
Freeman & Lindsay USA 1965	411	33 (8.0 per cent)	3.7 : 1
Williams et al. England 1966	—	66 (11.1 per cent*)	1.3 : 1
Bertoli et al. Italy 1967	84	10 (11.9 per cent)	1.5 : 1
Davis England 1967	222	15 (6.8 per cent)	0.9 : 1
Ibanez et al. USA 1967	562	53 (9.4 per cent)	1.1 : 1
Muller France 1969	600	31 (5.1 per cent)	1.1 : 1
Tubiana et al. France 1970	570	24 (4.2 per cent)	1.3 : 1
Franssila Finland 1971	229	10 (4.4 per cent)	1.5 : 1
Present series Sweden 1972	370	27 (7.3 per cent)	1.3 : 1

* 15 cases out of 135.

(No 2), suggest that the thyroid disease may occasionally be stationary for long time or even undergo spontaneous regression.

HISTOLOGICAL DIAGNOSIS

Amyloid is considered pathognomonic of medullary thyroid carcinoma. Vassar & Culling (1961) studied 45 examples of thyroid carcinoma other than of the medullary type; none of these showed amyloid. Williams et al. (1966) reported that they, too, were unable to find amyloid in thyroid tumours other than medullary carcinoma. This is consistent with the findings in the present material.

On the other hand, it is noteworthy, that Polliack & Freund (1970) reported one case of mixed papillary and follicular thyroid carcinoma, which contained amyloid as defined by current tinctorial criteria. Amyloid was also present in cervical lymph node metastases. The origin of the amyloid could not be determined, and the authors could not exclude that it might have been a manifestation of generalized amyloidosis. It was not reported whether there was any deposition within parts of the thyroid gland not involved by the

growth. Williams et al. (1966) found small amounts of amyloid remote from the tumour in 2 of their 67 cases of medullary thyroid carcinoma, in which the major site of deposition was the renal glomeruli. Zimmermann & Kracht (1969) also described one case of this tumour, where amyloid was demonstrated in the vessel walls in several parenchymatous organs. If medullary thyroid carcinoma could cause extra-tumoural deposition of amyloid, one would expect this to be more common than it is. It appears more likely that such amyloid deposits are related to factors other than the thyroid tumours.

It seems safe to state that amyloid is a characteristic feature of medullary carcinoma. This does not, however, imply that amyloid is a constant component of this neoplasm. One or more of the multiple thyroid tumours found in some of the familial cases reported here did not contain any amyloid. Metastases of the tumour may occasionally lack the substance. This suggests the existence of an amyloid-free type of medullary thyroid carcinoma. In this conjunction, it is noteworthy that the majority of the presumably corresponding tumours in the rat are devoid of amyloid (Lindsay et al. 1968).

In the presence of amyloid, the diagnosis of medullary thyroid carcinoma is easy. In the absence of this substance, however, its variable growth pattern may cause considerable diagnostic difficulties. It may suggest metastatic carcinoma and its ability to form solid, follicular or papillary structures may lead to confusion with other, biologically unrelated types of thyroid carcinoma. Tubiana et al. (1970) assumed the existence of a special variant of medullary carcinoma which lacked amyloid, but was otherwise morphologically similar to the amyloid-containing type. The authors emphasized that this variant differed from the amyloid-containing form in being more frequent among males than females, in that it lacked the clinical and pathological associations so frequently met with in the other type and ran a more malignant course, thus resembling that of anaplastic carcinoma. The authors based their definition of the "amyloid-free medullary carcinoma" on light microscopical, morphological criteria. Because of the variability of the growth pattern of medullary thyroid carcinoma, histological features alone seem to be rather unreliable tools for discriminating a presumable "amyloid-free medullary carcinoma" from thyroid neoplasms of different nature. Until an acceptable classification of such a new group of thyroid carcinoma can be established, we must try to find histological criteria which can be correlated with complementary distinctive features of different kinds, such as those revealed by electron-microscopical, histochemical or biochemical means, or a distinct clinical behaviour.

AMYLOID

The presence of amyloid in medullary thyroid carcinoma is enigmatic. It is still unknown, how and why this substance is formed.

There is, however, no doubt that the amyloid is formed within the tumour tissue. Apart from the 3 cases reported by Williams et al. (1966) and Zimmermann & Kracht (1969), amyloidosis, remote from the tumour has not been encountered in patients with medullary thyroid carcinoma.

Brandenburg (1954) appears to be the first to have suggested that the amyloid was produced by the epithelial tumour cells. This view has achieved further support by several, more recent studies, which have stressed the presence of material, histochemically and ultrastructurally similar to amyloid, within the cytoplasm of the tumour cells (Albores-Saavedra et al. 1964, Ibanez et al. 1967, Meyer & Abdel-Bari, 1968, Huang & McLeish 1968). These observations conflict with those of Lietz & Donath (1970), who defined a special stroma cell, resembling a smooth muscle cell which was thought might be the site of amyloid production in medullary carcinoma.

The findings in the present material suggest that epithelial tumour cells may be involved in amyloid formation. Tumour cells of undoubtedly epithelial nature have been observed, containing intracytoplasmic material with histochemical and ultrastructural features (Ljungberg & Boquist, unpublished observations) consistent with amyloid. Such cells are most abundant close to larger extracellular masses of amyloid. Sometimes, cells were seen which appeared to illustrate the gradual increase in the amount of intracellular amyloid that eventually occupied the whole cytoplasm with eccentric displacement of the nucleus. In the final stage, the cell disintegrated, leaving a rounded clump of amyloid, roughly the size of a tumour cell. This clump coalesced with neighbouring clumps to form large extracellular masses. Support for such a mode of amyloid formation is the observation that within some of the larger, extra-

cellular masses, the contours of the "sub-units" were still visible (see Fig. 6 c and d). This, however, appears not to be the sole mechanism of formation. In certain areas of the tumours the amyloid masses had a laminated texture, suggesting that the substance is laid down layer by layer. It is possible that other kinds of cells, such as the stroma cells of Lietz and Donath participate in this type of deposition.

On the other hand, it should be borne in mind that the presence of amyloid within a tumour cell need not mean that the substance is actually formed there. Though it appears unlikely, the possibility that the intracellular amyloid merely reflects a secondary intracellular inclusion of material primarily formed outside the cell, has not yet been dismissed.

It has been claimed that the substance might be related to colloid or thyroglobulin (Brandenburg 1954, Vassar & Culling 1961, Albores-Saavedra et al. 1964, Freeman & Lindsay 1965). Williams et al. (1966) pointed out that if this view was correct, follicular and papillary carcinoma may also be expected to be capable of forming amyloid. In addition, the tumour was found unable to capture and concentrate iodine, as demonstrated by autoradiographic studies using labelled iodine (Paper I). Recent studies have provided strong evidence that medullary thyroid carcinoma arises from non-follicular epithelial cells not concerned with thyroglobulin synthesis (Bussolati et al. 1969). There is thus no longer any basis for the "colloid or thyroglobulin"-theory as an explanation for the presence of amyloid in medullary carcinoma.

The chemical composition of amyloid in medullary carcinoma is not known; neither is it known why it occurs in this type of tumour. It has been proposed that it is most likely related to a high protein synthesis in the cells of the tumour, which are known to be able to produce large amounts of the poly-

peptide hormone calcitonin (Cunliffe et al. 1968, Meyer & Abdel-Bari 1968, Milhaud et al. 1968, Tashjian & Melvin 1968). Though there is no evidence that the amyloid is composed of calcitonin-polypeptide residues (Bussolati et al. 1969), it may possibly be built up of more or less altered precursor proteins of the secretory product. In this conjunction, it is noteworthy that certain other polypeptide-producing tumours, biologically closely related to medullary carcinoma, such as insuloma of pancreas and carcinoid, occasionally contain amyloid (Porta et al. 1962, Sterba 1968, Steiner 1969, Lindström & Ljungberg, unpublished observation). It should also be mentioned that Leedham & Pullock (1970) recently demonstrated amyloid in parathyroid glands of 9 out of 88 cases of primary hyperparathyroidism. The substance with the tinctorial characteristics of amyloid was situated in the lumina of follicular structures within the parenchyma. There were no signs of generalized amyloidosis in any of these patients. The position of amyloid in the lumina of follicles indicates that the epithelial cells are really involved in the formation of the substance.

GROWTH PATTERN AND PROGNOSIS

Judging from the findings in the present series, the histological structure does not by itself seem to be a reliable indicator of the further course of the disease. Patients who had survived for less than 2 years after operation have had tumours with principally the same histological pattern as those who had survived 20 years or more. Likewise, the presence of prominent cellular and nuclear polymorphism does not seem to reflect changes in degree of differentiation, but more likely degeneration in the tumour. In this respect, medullary thyroid carcinoma is similar to certain neuroectodermal tumours, such as phaeochromocytoma, neurofibroma and schwannoma.

The present observations also show that the amount of amyloid differs from one part of a tumour to another and may be abundant in metastases from a primary tumour containing only little or no amyloid. This is probably due to variability in the rate of formation of amyloid within different parts of the tumour as well as in different tumours and metastases. This view is consistent with the findings in the present series, where the amount of amyloid in the tumours varied considerably, regardless of duration of the thyroid disease prior to diagnosis. Though, in general, large tumours contained relatively more amyloid than did small ones, it seems rather unsafe to draw any conclusions with respect to a possible correlation between the actual growth rate of a given tumour and the relative amount of amyloid present in it. Nor is the amount of amyloid in the tumour a reliable indicator of the further course of the thyroid disease.

There was no good correlation between the degree of calcification in the tumours and the preoperative duration of the thyroid tumour as experienced by the patient, or the postoperative survival time.

Bone formation had occurred in the tumours at necropsy of 3 cases (Nos 2, 16 and 42). In 2 of these, the disease was known to have been present for at least 15 and 29 years. In the third, the duration was unknown, but the histological picture suggested regressive changes in the tumour. It is possible that the presence of bone foci may reflect an old age of the tumour, *viz.* a long duration of the thyroid disease. Whether this may also be prognostically significant, however, cannot be stated on the basis of this small number of cases.

In summary, no prognostically useful histological variable was found. These findings agree with those of Muller (1969), but conflict with the results of Ibanez et al. (1967)

who suggested that tumours containing large amounts of amyloid generally have a better prognosis. They also disagree with those of Williams et al. (1966), who stressed that a spindle cell pattern was associated with a bad and calcifications with a good prognosis. The importance of bone formation in the tumour deserves further study.

FAMILIAL MEDULLARY THYROID CARCINOMA. ASSOCIATION WITH PHAEOCHROMOCYTOMA AND OTHER PROLIFERATIVE DISORDERS OF NEUROEPITHELIAL TISSUES.

In the beginning of the 60s reports appeared on the occurrence of familial thyroid carcinoma and its frequent association with phaeochromocytoma (Cushman 1962, Friedell et al. 1962, Nourok 1964). In 1965, Schimke & Hartmann and Williams emphasized that when thyroid carcinoma was familial, it was most often of the medullary type and frequently associated with phaeochromocytoma. A great number of reports have since accumulated on familial medullary thyroid carcinoma, which has been seen in at least 110 members of 28 families (see Appendices I and II). In the majority of these families, there are also multiple cases of phaeochromocytoma, occurring alone or in association with the thyroid tumour. A small number of families with multiple cases of medullary thyroid carcinoma, but no apparent cases of phaeochromocytoma have also been described (see Appendix II). In some of the reports of these last mentioned families, however, the data about phaeochromocytoma are incomplete. It is possible that in some of these cases, adrenal tumours may have been present but escaped detection or had not yet developed by the time of the investigation. According to personal correspondence with Dr. Block, no phaeo-

chromocytomas had been detected by 1971 in the two families described by him and his associates in 1967. It is important to examine members of these families carefully for occult adrenal tumours and to follow them up for many years for phaeochromocytomas developing later in life. Not until this has been done will it be possible to decide whether this simple form of familial medullary thyroid carcinoma really does exist. It is difficult likewise to ascertain how often familial phaeochromocytoma occurs alone, i. e. without associated medullary thyroid carcinoma. Several accounts dealing with familial phaeochromocytoma have paid less attention to coexisting thyroid tumours, and in many earlier reports on familial phaeochromocytoma, thyroid abnormalities may not have been mentioned at all, because at that time the authors were not aware of the significance of the combination. On the other hand, a small number of cases of coexisting medullary thyroid carcinoma and phaeochromocytoma have also been reported, where familial occurrence seems to be lacking. In some of these cases, however, familial data have been incomplete as is the case in one patient of this series (Case No 12).

The genealogical trees in Fig. 2 of the three families included in this study confirm the previously postulated autosomal dominant mode of inheritance of this disease (Schimke & Hartmann 1965, Paper II). Consistent with such a mode of inheritance is the fact that approximately half the offspring from affected parents and married to normals are proven or probable cases. This is particularly striking in the American branch of Family I, studied by Melvin et al. (1971), where the diagnosis of the thyroid tumour had been made at a very early stage by means of hormone assays. Also, the majority of the affected subjects had a parent who was or probably had been affected. In addition, the sex distri-

bution is almost equal (Female/Male 1.3/1). A sex-linked mode of inheritance can be excluded, for there are examples of transmission from father to son. The data also indicate that the disorder in the families reported here is inherited with high penetrance, though the expression of the genetic defect varies. Of 39 affected subjects derived from all three families, 16 had both tumours, 20 thyroid carcinoma alone, and 3 phaeochromocytoma alone. An analysis of the families reported in the literature showed that the distribution of cases with only one of the two tumours or with both may vary from one family to another (See Appendix I). This variability of the phenotypic expression of the abnormal gene is possibly due to environmental or other genotypic factors. It is noteworthy, as can be seen from Table IV, that, taking all reported familial cases together, the total number of patients with both tumours constituted about one half of the total number of affected subjects, the other half consisting of cases with only one of the two tumours.

The age at onset of symptoms of the thyroid and adrenal tumours in the familial cases of the present series varied considerably, and did not differ conspicuously from that of the apparently sporadic cases. The mean age at the time of diagnosis was 46 years. The corresponding figure calculated from the previously reported cases is about 38 years, *i.e.* about one decade earlier than for apparently sporadic cases, as found in the major published series (Woolner et al. 1961, Ibanez et al. 1967). This difference may well be due to a greater alertness of patients belonging to families, where thyroid disorders are known to be common.

Bilateral and/or multiple gross thyroid tumours were present in 14 out of 16 familial cases in the present series. In the 2 remaining cases the glands were totally destroyed by tumour and definable growths were no longer

Table IV. Affected members of reported families with either medullary thyroid carcinoma or pheochromocytoma or with both, compared with corresponding apparently sporadic cases.

	Familial cases			Apparently sporadic cases		
	Both tumours	Thyroid tumour only	Phaeo-chromocytoma only	Both tumours	Thyroid tumour only	Phaeochromocytoma only
Total number	22 families 114 cases	6 families 21 cases	83 cases*	22 cases	268 cases	463 cases*
Both tumours	59 (52 %)			22		
Thyroid tumour only	30 (26 %)					
Phaeochromocytoma only	25 (22 %)					
Bilateral and/or multiple thyroid tumours	53 (60 %)	10 (48 %)		6 (27 %)	45 (17 %)	
Bilateral and/or multiple phaeochromocytomas	53 (60 %)		45 (54 %)	19 (86 %)		33 (7.5 %)

* Data are derived from Steiner et al. (1968).

evident. Bilateral and/or multiple pheochromocytomas occurred in 6 of 10 proven familial cases. This is similar to the findings in previously reported familial cases where this feature was present in about 60 per cent, but different from those in apparently non-familial tumours, collected from the literature in which this phenomenon was much rarer (17 per cent for the thyroid and 7.5 per cent for the adrenal tumours, see Table IV). It appears probable that the true frequency of multiple thyroidal tumours in familial cases is still higher, as the findings in the present material suggest. The low frequency of bilateral and/or multiple thyroid tumours in apparently non-familial cases with both types of tumours (27 per cent) may be explained by the fact that in many instances the site of the thyroid tumour(s) was not mentioned.

A characteristic finding in 10 of the familial cases and not seen in the apparently sporadic patients was the presence of multiple microscopical tumours and foci of proliferating nonfollicular epithelial cells in the spared thyroid parenchyma, remote from the gross tumours. Though some foci may be intra-

thyroidal metastases, the majority of these foci showed features of a gradual development from hyperplasia to gross tumours and undoubtedly represented very early stages of the tumours. The adrenal glands of two familial cases (Nos 3 and 9) were also remarkable in this respect. They showed a diffuse and multinodular enlargement of the medulla, suggesting an early stage in the development of pheochromocytoma. In summary, the findings support the view that both the thyroid and the adrenal tumours in familial cases have a multifocal origin and are preceded by hyperplastic changes of the progenitor cells from which these tumours arise.

The series studied in this paper includes one case of medullary thyroid carcinoma-pheochromocytoma associated with multiple mucosal tumours, confined to the tongue, lips and upper eye-lids, and with thick lips and thin limbs. Cumulative evidence of cases reported in the literature in the past few years suggests that various proliferative changes of the peripheral nervous system, sometimes associated with skeletal and soft-tissue deformities, form less frequent accompaniments of the "medul-

lary thyroid carcinoma-phaeochromocytoma"-syndrome. About 25 cases are known from the literature (cf Bartlett et al. 1971). This component of the syndrome occurs most frequently in apparently non-familial cases of medullary thyroid carcinoma with or without phaeochromocytoma, but has also been found in members of 3 families with such tumours (See Appendix I). Approximately one half of the patients have had both thyroid and adrenal tumours, the other half, thyroid cancer only. More than two thirds have had mucosal neuromas with the same facial appearance as found in the case of the present series. One third was found to have more generalized involvement of the peripheral nervous system, including proliferation of autonomic nerves to visceral organs and neuroma-like lesions of the posterior spinal nerve roots. Occasional patients were reported to have thickened peripheral somatic nerve trunks and nerve plexus and in 2 cases, the recurrent laryngeal and vagus nerves were reported to be greatly thickened (Bartlett et al. 1971). One third of the cases on record, showed a diffuse or segmental ganglioneuromatosis in the gastrointestinal tract, Auerbach's and Meissner's plexus being most often affected. Histologically, these lesions were described as a growth of nerve fibres, sometimes also with an increase in the number of ganglion cells within the plexus. Usually, the bowel wall was greatly thickened sometimes dilated and multiple diverticula in the small, and/or the large intestine were found. Occasional reports describe abnormal pigmentation of the skin (Ruppert et al. 1966, Johnston & Watson 1970, Pagès et al. 1970, Bartlett et al. 1971, Cunniffe et al. 1970) and sometimes also multiple cutaneous tumours mimicking von Recklinghausen's neurofibromatosis (Paper II, Schimke et al. 1968, Pagès et al. 1970, Franssila 1971). Various skeletal and soft-tissue abnormalities, including high-arched palate, pectus

excavatus, pes cavus and muscular weakness have also been found. The appearance of the lesions in the peripheral nervous system is frequently reported to have antedated that of medullary thyroid carcinoma and/or phaeochromocytoma. The mucosal neuromas have often been noticed at birth, as was the case in the patient in the present material. Likewise, the coexisting thyroid tumours appear to have become apparent at an earlier age than in familial and non-familial cases, where peripheral neural changes do not occur. Thus, the average age of the reported patients on admission to hospital was about 25 years, the youngest being a 4-year-old child, thyroidectomized for medullary carcinoma which had already metastasized to regional lymph nodes (Bartlett et al. 1971). This differs markedly from the average age at presentation for medullary carcinoma in general (40—50 years) and for the familial form without peripheral neural involvement (about 40 years).

OTHER ASSOCIATIONS

Diarrhoea was a principal complaint in about 30 per cent of the cases reported here. This proportion is consistent with the findings in other series (Williams 1966 b, Ibanez et al. 1967, Muller 1969). The stools were described as watery, sometimes frothy and irrepressible, especially after meals. Weight loss and dehydration were sometimes marked. Bernier et al. (1969) found the major abnormalities in the faeces to be increased content of water and electrolytes in 5 cases studied. The symptom seems to be most common in advanced cases and is considered to vary with the size of the tumour. This is illustrated by case 39, in which removal of a large part of metastatic tumour was followed by a temporary disappearance of the diarrhoea. In some patients, however, the diarrhoea had accompanied the

onset of the goitre. It is possible, that in such cases, the malignant disease has already metastasized. Fig. 3 shows that 11 of the 14 cases with this symptom, died with signs of dissemination of the thyroid carcinoma. Facial rubeosis may be present in patients with medullary carcinoma and diarrhoea, as found in 1 of 12 patients studied by Williams (1966 b). In the present series, one patient (No 30) was reported to have been red-faced. Two patients had been operated for *peptic ulcer*, in one case (No 39) a large ulcer of the stomach was found at necropsy and in another 2 peptic ulcers had been diagnosed radiographically. Two other patients had had symptoms of gastritis requiring medical treatment. Wallace et al. (1970) emphasized that peptic ulcers may be an additional specific accompaniment of medullary thyroid carcinoma. The occurrence of 5 proven cases of peptic ulcer in the present material (about 12 per cent) does not allow any definite conclusion in this respect. It is noteworthy that in one of the cases (No 38), hyperparathyroidism with a parathyroid adenoma, weighing 130 g was diagnosed several years after the ulcer had been detected.

In 1959, Hökfelt et al. reported one case of *Cushing's syndrome* associated with carcinoma of the thyroid. Later, when reviewing the histology of this and other previously reported cases with this combination, Williams et al. (1968 a) found that in the majority of the cases, the thyroid tumours were of the medullary type. Pathologically, the adrenal glands show bilateral cortical hyperplasia. Ectopic production of ACTH-like substances have been demonstrated by bioassay in several of these instances (Goldberg & McNeil 1967, Donahower et al. 1968, Szijj et al. 1969, Vague et al. 1971).

It is well known that there is an increased incidence of *parathyroid adenoma or hyperplasia* in association with either the inherited

syndrome or sporadic medullary carcinoma (Steiner et al. 1968). It is difficult to assess the true incidence of this abnormality from the literature. At least 30 cases have been recorded. It is possible, that parathyroid changes are much more common in patients with medullary carcinoma than was originally supposed, for in 2 families, carefully examined for parathyroid changes (Melvin et al. 1971, Catalona et al. 1971), enlargement of the parathyroids was found respectively in 9 of 11 and in 6 of 12 cases. The histological diagnosis of the parathyroid changes in the reported cases had been "adenoma", "hyperplasia" or simply "enlargement". In several instances the authors stated that it was not possible to decide whether the changes should be interpreted as adenoma or hyperplasia. Most of the enlarged glands had been built up mainly of chief cells, but there were also examples of glands consisting mainly of waterclear cells or mixtures of different cell types. In about one third of the reported cases serum calcium levels had been elevated and usually returned to normal after removal of the enlarged parathyroid gland(s). In some patients renal stones had been present. In case No 38 of the present material, the diagnosis had been primary hyperparathyroidism. At operation, an parathyroid adenoma (130 g) was removed together with one thyroid lobe which, incidentally was found to be involved by a medullary carcinoma.

NATURE OF MEDULLARY THYROID CARCINOMA

Since the definition of medullary thyroid carcinoma in 1959 by Hazard et al., it became widely accepted as a distinct entity. But it was not until about one decade later that cumulative evidence suggested that the tumour had also a distinct histogenesis. On the

basis of comparative morphological studies, Williams (1966 a) thought that medullary thyroid carcinoma was derived from parafollicular cells, the calcitonin producing cells of the thyroid gland.

These cells have long been known in mammalian thyroid under various names, such as "parenchymatous cells" (Baber 1876), "cytoplasmareichen Zellen" (Hürthle 1894) and "parafollicular cells" (Nonidez 1931/32). They are now generally regarded as a normal endocrine cell constituent of mammalian thyroid and have been found to be responsible for the production of the polypeptide hormone calcitonin (Bussolati & Pearse 1967, Kalina & Pearse 1971). Calcitonin was discovered in 1962 by Copp et al. Hirsch et al. (1963) showed that in mammals, the thyroid gland was the principal site of calcitonin production. The hormone has been isolated from several species and purified. It is a 32 amino acid polypeptide with 1—7 intrachain disulphide bridge at the amino terminal end and a proline amide at the carboxyl end of the molecule. There are minor differences in the structure between species. The hormone is involved in calcium homeostasis, though its physiological significance is not properly understood. When administered to experimental animals it may produce hypocalcaemia and hypophosphataemia. In man the serum changes are less striking (for review, see Hirsch 1971).

In the current literature the calcitonin producing cells have frequently been referred to as "parafollicular cells" although it is now realized that this term is inadequate. The cells may also have other topographical relations to the thyroid follicles and they have been demonstrated in the parathyroid glands of certain mammals. Furthermore, in avian and amphibian species, these cells are chiefly incorporated within the ultimobranchial body, which is a separate structure lying outside

the thyroid. The term "C-cells", introduced by Pearse in 1966, defines these cells according to their function of storing and secreting calcitonin and avoids the problem of giving them an adequate topographical name. The C-cells are readily recognized by well established histochemical techniques in the thyroid gland of many animals (for review, see Lietz 1971). But they are difficult to detect in man, mainly due to their rarity and patchy distribution. Sugiyama (1967), in a study of 326 human thyroid glands, could detect these cells in only two cases. Teitelbaum et al. (1971) found areas suggesting the presence of C-cells when more than 200 random sections from one thyroid lobe had been examined histologically.

The proposal by Williams (1966 a) that the tumour could arise from C-cells has since received further support. Large amounts of *calcitonin* (as much as 5,000 times the amount in the normal gland) have been extracted from medullary tumours (Cunliffe et al. 1968), the hormone also being present in increased concentration in the circulation. The tumour has been found to contain cells with ultrastructural characteristics also found in normal mammalian C-cells (Gonzalez-Licca et al. 1968, Meyer & Abdel-Bari, 1968). Cells in the tumour may display histochemical features which are also found in normal C-cells, including argyrophilia (De Grandi 1970) and so-called masked metachromasia, *i.e.* a metachromatic reaction after staining with certain basic dyes such as toluidine blue, Azur A and pseudisocyanin preceded by hydrolysis in strong acid (Solcia et al. 1968). Finally, calcitonin has been demonstrated in a certain proportion of the tumour cells by immunohistochemical methods (Bussolati et al. 1969).

The C-cells as well as medullary carcinoma are sensitive to a high calcium stimulus, which increases secretion of hormone. This

characteristic has recently been used clinically to diagnose the tumour at an early stage by means of hormone assays during calcium infusion (Melyin et al. 1971).

Medullary carcinoma may also contain small amounts of certain *monoamines*, as revealed by chemical and histochemical studies (Paper III). Chemical analysis disclosed small amounts of 5-hydroxytryptamine. The histochemical study of the medullary tumours with the method of Falck-Hillarp demonstrated a cellular system with a specific cytoplasmic fluorescence characteristic of compounds such as the primary catecholamines, while no fluorescence characteristic of 5-hydroxytryptamine could be demonstrated. As to the reason why the results of the chemical analysis were not in accord with those of the histochemical study, several explanations may be considered. The fluorophore may be derived from other substances than catecholamines, but closely related to these. Alternatively, the fluorescence was caused by some of the known catecholamines which were so firmly bound in the malignant cell that they could not be extracted with the method used for chemical analysis. Medullary carcinoma has also been found to contain a small population of cells which differs from the main mass of cells in being argentaffin and in having a characteristic spider-like shape (Papers IV and V). Argentaffin cells, chiefly in parafollicular position, have also been found in non-tumorous thyroid tissue and in parathyroid glands in cases of medullary carcinoma. This second population of non-follicular cells are morphologically, topographically and histochemically distinct from the C-cells. Since argentaffin cells were also found in metastases of medullary carcinoma (Papers IV and V), these cells seem to constitute an integral part of the tumour, being the malignant counterparts to the argentaffin cells found in thyroid tissue not involved by the

tumour. Their biological relationship to the C-cells is not known. They may represent "functional variants" of the C-cells, but their characteristic shape and distribution may also suggest a distinct type of cell with different functions. It is not known whether the fluorescent cells found with Falck-Hillarp's method and the above-mentioned argentaffin cells are identical. If the fluorescence found is due to firmly bound monoamines, these substances might perhaps also be able to reduce silver salt and render the cells argentaffin under the conditions used in the silver salt procedure. Storage of endogenous monoamines and/or uptake of their precursors, decarboxylation of the latter as well as storage of the amines thus formed, are common characteristics of a number of polypeptide hormone-producing cells, including cells of the pancreatic islets, argyrophil and enterochromaffin cells of the gut, cells of the adenohypophysis and also C-cells (See Pearse 1969). Falck et al. (1964) were the first to demonstrate this in C-cells of the sheep thyroid gland. Cells in the adrenal medulla and carotid body, melanocytes and Feyrter's clear cells of bronchial mucosa also possess such characteristics, but the postulation that these cells also produce polypeptide hormones (Pearse 1969), abides confirmation. The physiological role of the monoamine mechanisms in the C-cells is unknown. They were not found to be influenced by induced changes in calcitonin metabolism (Kruse 1971). It is not surprising that medullary thyroid carcinoma, which at least partially originates from C-cells, may also contain monoamines. Human C-cells do not normally contain monoamines, but can take up their precursors (Englund et al. 1972). In view of the common coexistence of pheochromocytoma in patients with medullary thyroid carcinoma, the presence of amines in the thyroid tumours may reflect such an uptake mechanism. It is also possible, however, that

the malignant cell has achieved the full capacity of endogenous formation of these amines.

Medullary thyroid carcinoma has been found to contain a number of other biologically active substances. Its capacity of forming *ACTH-like substances* with the production of Cushing's syndrome has been referred to previously in this paper. In 1968, Williams et al. found appreciable amounts of *prostaglandins E₂ and F₂α*, which are biologically active fatty acids, in 4 out of 7 examples of the tumour and raised blood levels were detected in 2. Later, the same authors (Sandler et al. 1969) found high levels of these substances in other amine and/or polypeptide producing tumours such as ganglioneuroma, neuroblastoma, phaeochromocytoma, pancreatic islet tumour and bronchial carcinoid, but not in ileal carcinoid. Raised *histaminase activity* has recently been demonstrated both in medullary carcinoma and in serum of patients with this tumour (Baylin et al. 1970 and 1972). Raised serum level, however, was not a constant feature, for in 12 out of 33 cases studied by these authors, the serum activity was normal.

CONSIDERATIONS ON THE PATHO-GENESIS AND AETIOLOGY OF MEDULLARY THYROID CARCINOMA AND ITS ASSOCIATED DISORDERS

Sipple (1961) was one of the first to offer a tentative explanation of the association between medullary thyroid carcinoma and phaeochromocytoma. He felt that the thyroid tumour might be secondary to prolonged catecholamine excess. To-day, this explanation appears less likely. A number of cases have been observed in which the medullary thyroid carcinoma appeared before the symptoms of phaeochromocytoma. In addition, in families

with both tumours, some members have had the thyroid tumour but apparently not phaeochromocytoma.

Attempt has been made to find an embryological explanation for this association. It has thus been ascribed to an abnormality in the control of differentiation of a single cellular system, most probably derived from neuroectodermal cells and the name "Chromaffinomatosis" has been proposed for the syndrome (Paper II). This concept was based on the following observations: 1. the tendency of the syndrome to be inherited, 2. the multifocal character of the adrenal and possibly also the thyroid tumours, 3. the occurrence of other proliferative changes of neuroepithelial tissue in the patients, 4. the occurrence of chromaffin cells in the thyroid tumours (Paper IV) and 5. the morphological similarity between the thyroid and adrenal tumours.

New evidence has appeared which lends further support to this view. While the multifocal nature of the adrenal tumours in these patients is beyond any doubt, this may be debatable concerning the thyroid tumours. Since the thyroid tumours are malignant and apt to metastasize, it might be argued that the multiple tumours and foci of proliferating non-follicular cells are not multicentric growths but merely a result of intrathyroidal metastatic spread of cancer cells. Several findings, however, support the view that the majority of these foci represent a multifocal manifestation of the thyroid disease.

1. There does not seem to be any striking difference between the familial and the sporadic variety of medullary thyroid carcinoma as regards natural behaviour, including the proneness of extrathyroidal metastatic spread. Provided the multiple thyroid tumours so often found in the familial cases, do represent metastases, multiple tumours would have been expected more often in the sporadic cases,

without association with phaeochromocytoma. 2. Many microscopical foci of proliferating non-follicular cells found in the parenchyma, remote from the gross tumours in the familial cases, are topographically related to the follicles in a way which is more compatible with foci of C-cells which have undergone hypertrophic and hyperplastic changes *in situ* than with foci of metastasizing cancer cells. The appearance of these foci of cells illustrates the gradual development from hyperplasia to true tumours with clearly neoplastic features, such as nodular appearance and definite destruction of follicles. When these foci become larger, however, it is no longer possible to study the mechanism of their origin. The hyperplastic foci were found in patients, who were adults. This means that the thyroid disease is still active at this age and that the patient is constantly apt to develop new tumours.

3. Experimental studies in animals show that a long-standing high calcium stimulus can induce hypertrophy and hyperplasia in the C-cells (Walker 1966, Rohr & Hasler 1968, Capen & Young 1969). The morphological appearance of the changes (Kameda 1970) may strikingly resemble the hyperplastic foci of cells found in the human cases studied here. Furthermore, hypertrophy and focal nodular hyperplasia with a tumour-like proliferation of the C-cells have also been observed in one human case with primary hyperparathyroidism and longstanding hypercalcaemia (Paper VI). The morphological similarity between the thyroid change in this case and most of the microscopical foci of cells found in the familial cases of medullary carcinoma is evident (see Paper VI).

The embryogenesis of the progenitor cells from which medullary thyroid carcinoma arises has been debated. Godwin (1936/37) suggested that the parafollicular cells, now known as C-cells, develop from the ultimobranchial

body, and are therefore of entodermal origin. This assumption was supported by studies of Pearse & Carnevali (1967). The demonstration of monoamines in cells of medullary thyroid carcinoma led to the suggestion that the tumour was at least partially derived from neuroectodermal cells and was thus closely related to phaeochromocytoma (Papers III and IV). There is new evidence that the C-cells develop primarily from *crista neuralis* (Le Douarin & Le Lièvre 1970, Pearse & Polak 1971).

The increasing evidence that medullary thyroid carcinoma is a neuroepithelioma of the thyroid provides the missing link, which assembles most of the manifestations of the "medullary thyroid carcinoma-pheochromocytoma"-syndrome under a common pathogenetic denominator. Thus, the various proliferative changes of the peripheral nervous system as well as abnormalities in pigmentation of the skin, sometimes also encountered in these patients, may well be thought to be caused by the same principle.

It is noteworthy that when neuroma and ganglioneuromatosis occur in a patient with the disease, these lesions tend to develop early in life and are sometimes evident at birth. The thyroid and adrenal tumours also seem to develop earlier than in patients without peripheral neural lesions. This suggests that the common causal principle has exerted its effects during the foetal period of life in these cases.

The rare occurrence of various skeletal and soft tissue deformities in patients with the syndrome is more difficult to explain, though some, such as the occurrence of thick lips may be secondarily related to the proliferation of nerve tissue.

The cause of diarrhoea in patients with medullary carcinoma is not known with certainty though several possible explanations have been offered. Both 5-hydroxytryptamine

and prostaglandins, sometimes extracted from the tumours, are capable of producing this symptom, and facial flushing, which may occasionally be an accompanying feature (Moertel et al. 1965, Williams et al. 1968 b, Horton 1969). Another possibility is that kinins, found in carcinoid tumours, are also present in medullary thyroid carcinoma and capable, alone or synergistically, of producing these symptoms. It is further possible, that the diarrhoea found in patients with accompanying peripheral nervous changes is due to neurogenous functional disorders of the gastro-intestinal tract.

Parathyroid hyperplasia or adenoma in patients with medullary thyroid carcinoma have been claimed to be a compensatory response to increased circulating calcitonin formed by the thyroid tumour (Schimke et al. 1968, Steiner et al. 1968, Bartlett et al. 1971, Catalona et al. 1971). Melvin et al. (1971) found raised serum levels of parathyroid hormone in several patients with the thyroid tumour. Overstimulated parathyroid glands may become autonomous and then produce abnormally large amounts of hormone. This may explain the hypercalcaemia found in some patients with the thyroid tumours. Though this view may be correct in some instances, the reverse order of the patho-physiological events must also be considered. A prolonged high serum calcium level can induce hypertrophy and hyperplasia of the C-cells in experimental animals (see Paper VI). Hypertrophy and hyperplasia with a tumour-like proliferation of the C-cells has been observed in a patient with primary hyperparathyroidism and longstanding hypercalcaemia (Paper VI).

In that case the basic disorder appears to have been the parathyroid disease. The change in the thyroid strikingly resembled the early stages of medullary carcinoma, as found in the familial cases of the present material. The findings suggest that a long-standing hypercalcaemic state may be a causal factor in some C-cell tumours. The patient in Case 38 was noteworthy in that the thyroid carcinoma was discovered incidentally at operation for a remarkably large parathyroid adenoma (weighing 130 g) which had produced a clinical picture compatible with primary hyperparathyroidism. This view gains further strength from the observation that tumours resembling human medullary carcinoma are frequently found in bulls, but not at all in cows (Krook et al. 1969, Wilkie & Krook 1970). These authors suggested that high level of dietary calcium might be the most important aetiological factor in the development of these tumours in bulls, while the absence of the tumours in cows was thought to be due to a lesser functional strain on the C-cells in animals using calcium for lactation and foetal growth. Wilkie & Krook (1970) found phaeochromocytoma to be a common accompaniment of the thyroid tumours in bulls and thought that environmental factors resulting in thyroid neoplasms might also influence adrenal medullary cells. In this conjunction it might be worth while to recall that Kaplan et al. (1970) reported the presence of a hypocalcaemic principle in adrenal medulla of the pig, which was indistinguishable by radioimmunoassay from porcine thyroid calcitonin.

VI. SUMMARY AND CONCLUSIONS

(including Papers I—VI)

Clinical, pathological, histochemical as well as biochemical findings in 42 cases of medullary thyroid carcinoma, including 16 familial cases are reported. The observations of interest are summarized below.

1. Medullary thyroid carcinoma was a rare tumour, comprising about 7 per cent in a series consisting of 370 studied cases of thyroid carcinoma. Its sex distribution was almost equal (Females/Males: 1.3/1). Its clinical onset and course varied. It metastasized early to regional lymph nodes (in about 50 per cent of the cases at the first operation) but its future course was often slow. Accompanying diarrhoea (present at operation or developing later in about 30 per cent) appeared to be related to dissemination of the malignant disease. The frequency of peptic ulcers (about 12 per cent) was emphasized.
2. Its histological growth pattern showed wide variations, which were described in detail. Amyloid was a characteristic feature, but possibly not invariable. Autoradiographic studies after administration of ^{131}I and ^{125}I to patients failed to demonstrate any radioiodine within the amyloid or tumour cells and lent no support to the theory that amyloid in these tumours could be altered thyroglobulin (Paper I).
3. Three families with the "medullary thyroid carcinoma-phaeochromocytoma"-syndrome were studied, one in greater detail (Paper II). The mode of inheritance was autosomal and dominant. In the familial syndrome, the thyroid tumours, like the phaeochromocytomas were often multifocal.
4. 5-Hydroxytryptamine was demonstrated chemically in thyroid tumours as well as in phaeochromocytomas from patients with the familial form of the syndrome. Histochemical analysis according to the method of Falck-Hillarp disclosed cells in the thyroid tumours displaying a fluorescence compatible with catecholamines or closely related substances. Possible explanations for the discrepancy between the chemical and histochemical findings are offered (Paper III). The thyroid tumours resembled phaeochromocytoma both morphologically and histochemically. Their association with this tumour and other proliferative disorders of neuroectodermal tissues as well as their tendency to be inherited, and then often concurrently with these disorders, support the view that they are neuroepithelial tumours and point to a common causal principle of the syndrome (Papers II and IV).
5. Two types of cells were demonstrated in familial as well as in sporadic cases of medullary carcinoma and in metastases from the tumour (Papers IV and V). One type of cell was unevenly distributed, it had a spider-like shape, was argentaffin and

chromaffin and showed an orthochromatic reaction when stained with certain cationic, aniline dyes. The other, constituting the major part of the tumour tissue, showed characteristics consistent with C-cells, being non-argentaffin but argyrophil and metachromatic. Staining with cresyl violet was found valuable for demonstrating human C-cells (Paper V). The non-neoplastic counterpart to the argentaffin "spider-cell" found in the tumours was discovered in thyroid and parathyroid tissue (Paper V).

The relationship between the argentaffin cell and the C-cell is discussed (Paper V).

6. Although parathyroid changes found in some patients with medullary thyroid carcinoma may be secondary to a high calcitonin secretion from the thyroid tumour, the possibility must also be considered that parathyroid hyperfunction or other conditions associated with long-standing hypercalcaemia may play a causal role in the development of some of the thyroid tumours (Paper VI).

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APPENDICES

APPENDIX I

Description of affected members of 22 reported families with coexistent medullary thyroid carcinoma and phaeochromocytoma, including present cases.

Case No.	Authors	Age at presentation and sex	Thyroid tumour	Adrenal tumour	Comment
1	De Graeff et al. (1959)	27 M	+	Bilateral	2 parathyroid chief cell adenomas
2		52 M	—	Bilateral	
3	Smits & Hui-zinga (1961)	62 M	+	Bilateral	
4		31 M		Bilateral	
5		30 M		One in left adrenal	
6		15 F		One in right adrenal	
7	Cushman (1962)	55 M	Probable bilateral	One in right adrenal	One parathyroid adenoma
8		32 M	+	One in left adrenal	
9		12 F	+	—	
10	Finegold & Haddad (1963)	27 M	+	Bilateral	2 parathyroid chief cell adenomas
11		52 M	?	Bilateral	
12	Grunstein &	28 F	+	Bilateral	
13	Finkelstein (1962)	34 M	—	One in right adrenal	
14	Manning et al. (1963)	18 F	Bilateral	Bilateral, multiple extraadrenal tumours	Parathyroid chief cell hyperplasia of 3 glands
15		? F	?	Bilateral	
16		? M	?	Bilateral	
17	Nourok (1964)	25 F	Bilateral	Bilateral, 2 in right, 3 in left adrenal	
18		30 F	One in left lobe	Bilateral	
19	Schimke & Hartmann (1965)	18 F	+	Bilateral	
20		22 F	+	Bilateral, 2 in left, one in right adrenal	
21		39 M	Bilateral	Bilateral	

Appendix I—*continued*

Case No.	Authors	Age at presentation and sex		Thyroid tumour	Adrenal tumour	Comment
22	Zanen & Van Leeuwen (1965)	25	M	+	Bilateral	Other family members affected. Details not mentioned
23	Williams & Pollock (1966)	19	F	One in left lobe	Bilateral	Mucosal neuromas, intestinal ganglio-neuromatosis
24		21	M	+	?	Probable mucosal neuromas
25	Aubert et al. (1967)	30	F	Bilateral	Bilateral, one in left, 2 in right adrenal	
26		28	F	+	—	
27	Vries et al. (1967)	25	M	Bilateral	Bilateral	2 enlarged parathyroid glands
28		37	M	+	One in right adrenal	3 parathyroid chief cell adenomas
29		42	F	Bilateral	Bilateral	
30		53	F	+	One in left, 2 in right adrenal	One parathyroid chief cell adenoma Nephrocalcinosis
31	Huang & McLeish (1968)	66	M	Bilateral	Bilateral, ganglio-neuroma in one tumour	
32		32	M	+	More than 10 tumours in right, normal left adrenal	
33		25	F	+	Bilateral	
34		?	F	?	+	
35		?	F	?	+	
36		?	M	?	+	
37		?	M	?	+	
38		?	M	?	+	
39		?	M	?	+	
40	Sarosi & Doe (1968)	54	M	Bilateral	Bilateral; extra-adrenal ganglio-neuroma	2 parathyroid adenomas
41		43	F	+	?	
42		40	M	—	One adrenal tumour	
43	Steiner et al. (1968)	51	M	?	Bilateral	
44		59	M	+	One in left adrenal	
45		41	M	Bilateral	Bilateral	Cushing's syndrome
46		23	M	?	Bilateral	
47		49	F	?	Bilateral	
48		32	M	?	Bilateral	
49		36	F	Bilateral	Bilateral	Parathyroid chief cell hyperplasia
50		33	M	Bilateral	Bilateral	
51		19	F	?	Bilateral	
52		24	M	—	Bilateral	
53		17	F	+	—	Parathyroid chief cell hyperplasia

Appendix I—continued

Case No.	Authors	Age at presentation and sex	Thyroid tumour	Adrenal tumour	Comment
54	Pagès et al. (1970)	60 F	+	One in left adrenal	Cutaneous neurofibromatosis, hyper-pigmented skin spots. Four relatives had cutaneous neurofibromatosis and one cerebellar astrocytoma
55		? M	—	One in right adrenal	
56	Bartlett et al. (1971)	23 F	Bilateral	One in left adrenal	Mucosal neuromas, intestinal gangli-neuromatosis. Renal stones
57		37 M	+	One in right adrenal	Mucosal neuromas, intestinal gangli-neuromatosis
58		35 M	Bilateral	Bilateral	One parathyroid adenoma, mucosal neuromas, intestinal ganglioneuroma-tosis
59		4 M	Bilateral	—	Café-au-lait skin spots, mucosal neuro-mas, intestinal ganglioneuromatosis, thickened recurrent laryngeal and vagus nerves. One relative had mucosal neuromas and symptoms of pheochromocytoma
60	Catalona et al. (1971)	44 F	Bilateral	Bilateral	One adenomatous, 2 hyperplastic para-thyroid glands
61		15 F	Bilateral	One in right adrenal	
62		38 F	Bilateral	Bilateral	
63		63 F	Bilateral	Bilateral	
64		18 F	Bilateral	Bilateral	
65		46 M	Bilateral	2 in right adrenal	Renal stones
66		33 M	Bilateral	Bilateral	Parathyroid hyperplasia
67		31 M	Bilateral	Bilateral	Parathyroid hyperplasia
68		37 M	Bilateral	One in right adrenal	2 parathyroid adenomas
69		47 F	Multiple in left lobe	Bilateral	Parathyroid hyperplasia
70		41 M	Bilateral	—	
71		29 M	Bilateral	—	
72		27 M	Bilateral	—	2 parathyroid adenomas
73		56 M	Bilateral	—	
74	Melvin et al. (1971)	37 F	Bilateral	One in right adrenal	Parathyroid hyperplasia
75		34 F	Bilateral	—	Parathyroid hyperplasia
76	Branch of pre-sent family I	24 F	Bilateral	—	Parathyroid hyperplasia
77		27 F	Bilateral	Bilateral	Parathyroid hyperplasia
78		39 F	Bilateral	Bilateral	Parathyroid hyperplasia
79		29 M	Bilateral	—	Parathyroid hyperplasia
80		61 M	Bilateral	One in left adrenal	Parathyroid hyperplasia
81		29 M	Bilateral	—	
82		64 M	Bilateral	—	Parathyroid hyperplasia
83		30 M	Bilateral	—	Parathyroid hyperplasia
84		36 M	Bilateral	—	

Case No.	Authors	Age at presentation and sex	Thyroid tumour	Adrenal tumour	Comment
85		26 M	—	One in left adrenal	
86		? M	+	+	
87		? M	+	—	
88		? M	+	—	
89		? M	+	—	
90		? F	+	—	
91		? F	+	—	
92		? F	+	—	
93		? F	+	—	
94	Sato et al. (1971)	43 F	+	Bilateral, multiple in both adrenals	
95		21 F	+	Bilateral, multiple in left adrenal	
96 (1***)	Present Family I	75 F	+	?	Cardiovascular attacks suggesting pheochromocytoma
97 (2)		79 M	Bilateral	Bilateral	
98 (3)		42 F	Bilateral	Multiple minute nodules in right adrenal	Rena stones. Cutaneous fibroma-like tumours
99 (4)		52 F	Bilateral	One in right adrenal	Parathyroid hyperplasia. Cutaneous fibroma-like tumours
100 (5)		39 F	Bilateral	One in right adrenal	
101 (6)		50 M	Bilateral	—	Cutaneous fibroma-like tumours
102 (7)		48 M	Bilateral	—	Cutaneous fibroma-like tumours
103 (8)		44 F	Bilateral	One in left adrenal	Cutaneous fibroma-like tumours
104 (9)		63 F	Bilateral	Diffuse and multi-nodular enlargement of right adrenal medulla	
105 (10)		31 F	Bilateral	—	
106 (11)		36 F	Bilateral	Bilateral	
107		51 M	?	One in right adrenal	
108		38 F	?	Adrenal phaeo-chromocytoma	Died after parturition
109 (12)	Present Family II	48 F	Bilateral	One in left adrenal	
110 (13)		51 M	Bilateral	—	
111 (14)		32 F	Bilateral	2 in each adrenal	One parathyroid adenoma
112		39 F	+	Bilateral	
113 (15)	Present Family III	40 F	+	?	
114 (16)		45 M	Bilateral	2 in left adrenal	

* Revised diagnosis on review by Williams (1965).

** Revised diagnosis on review by Shimke & Hartmann (1965).

*** Number in brackets is case number in the present material.

APPENDIX II

Description of affected members of 6 reported families with medullary thyroid carcinoma only.

Case No.	Authors	Age at presentation and sex	Site of thyroid tumour	Comment
1	Friedell et al (1962).	21 F	Bilateral	One relative died from thyroid cancer
2		24 M	Bilateral	
3	Block et al. (1967) Family I	? M	+	Bilateral involvement in at least 4 members. One affected member had parathyroid adenoma
4		? F	+	
5		? F	+	
6		? F	+	
7		? F	+	
8		? F	+	
9		? F	+	
10	Block et al. (1967) Family II	? F	+	
11		? F	+	
12		? F	+	
13	Schimke & Hartmann (1965)	20 F	Bilateral	Parathyroid hyperplasia
14		43 F	Bilateral	
15	Gonzalez-Licea et al. (1968)	41 M	Bilateral	Parathyroid hyperplasia
16	Muller (1969)	16 M	+	
17		? M	+	
18	Weiler et al. (1969)	15 F	+	One in the left lobe 2 in left lobe
19	Tubiana et al. (1970)	22 F		
20		19 F		
21		52 F	+	

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